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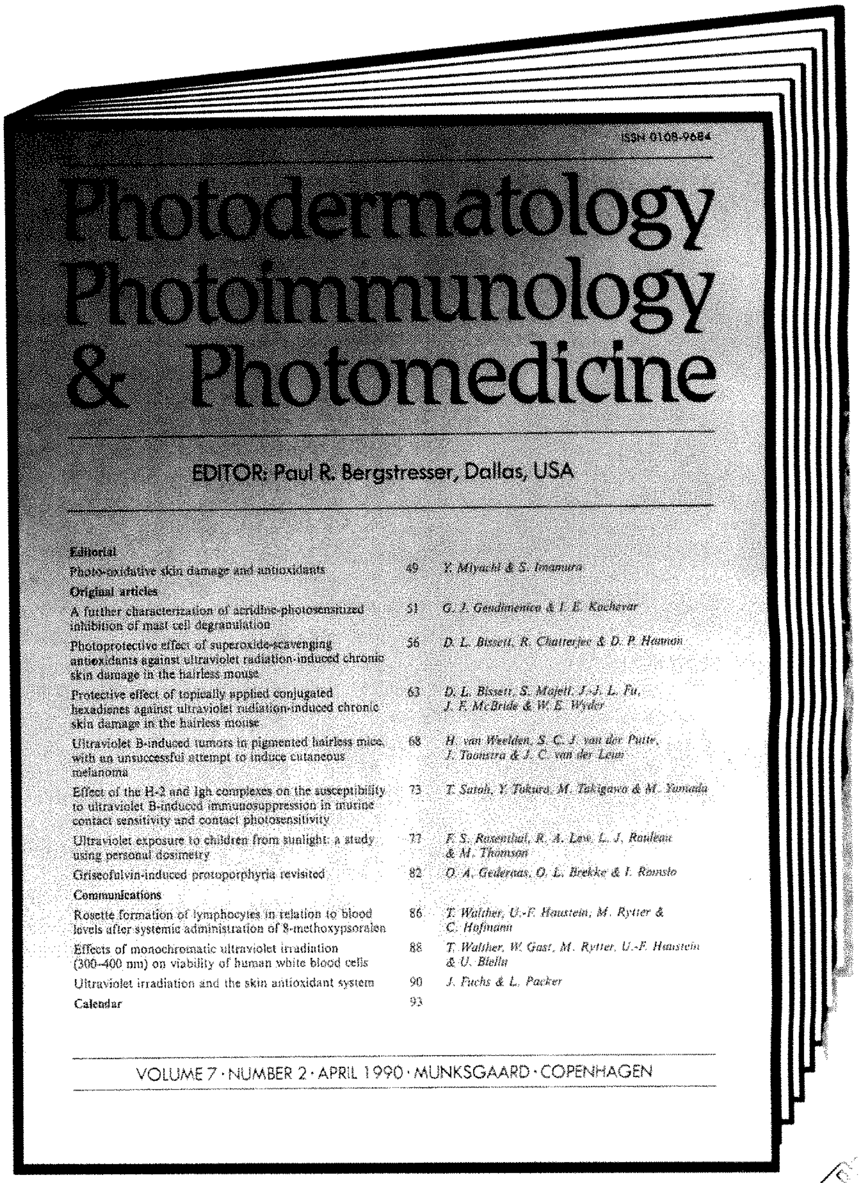

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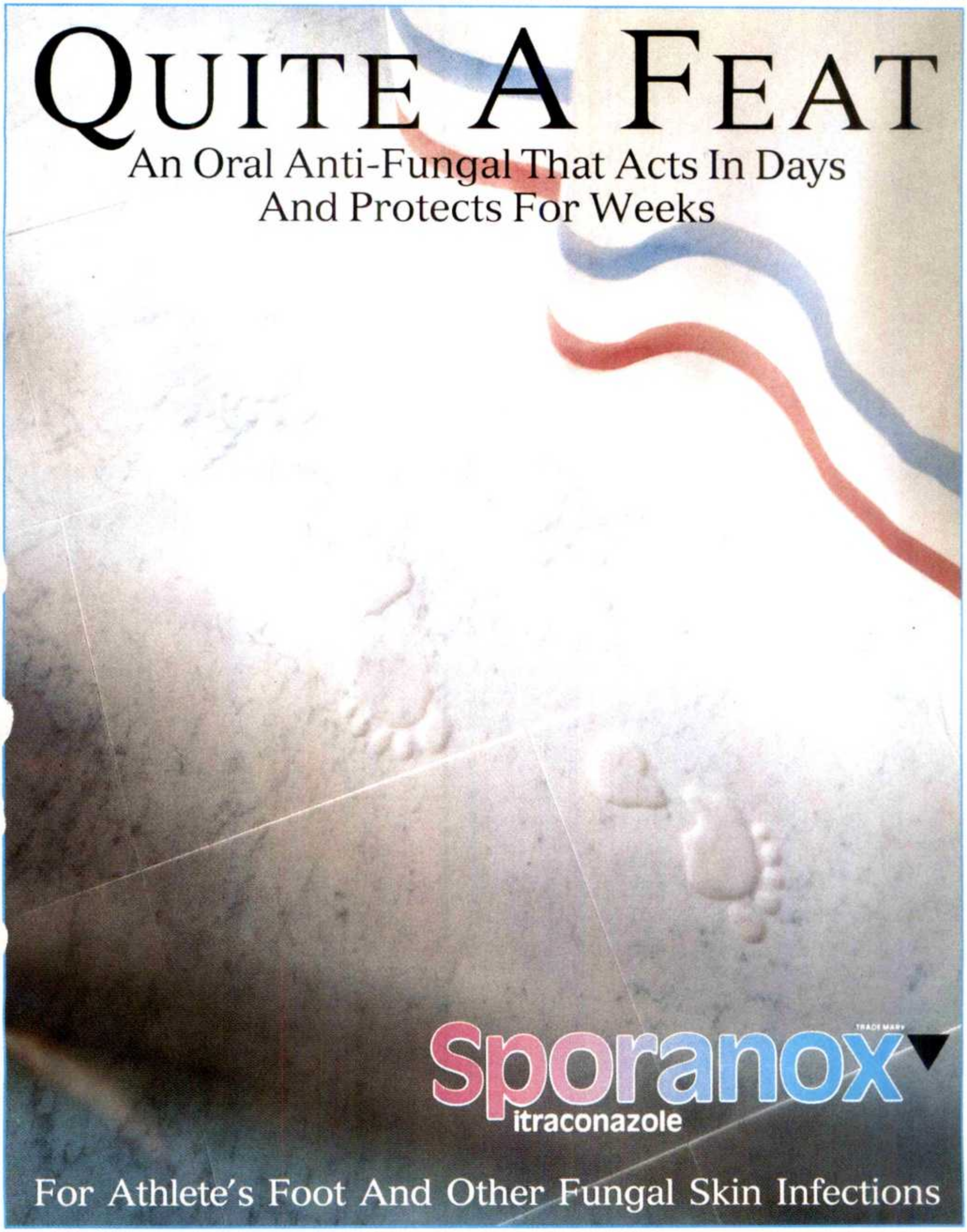
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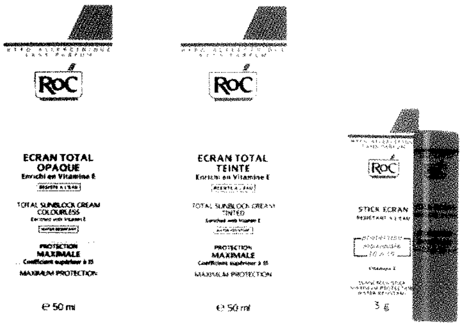

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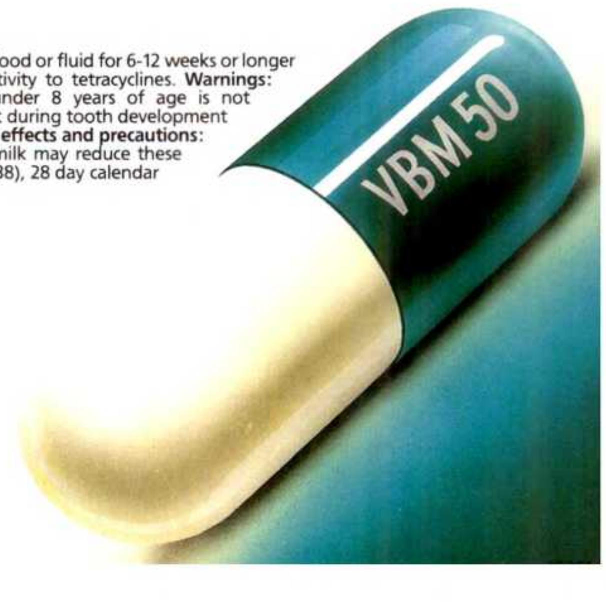
1. Harrison, P.V., (1988) Clin. Exp. Dermatol 13, 242-244



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REVIEW

Cutaneous lymphoproliferative disorders: an assessment of recent investigative techniques

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For nearly 100 years, the diagnosis of cutaneous lymphoproliferative disorders has been achieved primarily by a combination of clinical acumen and the assessment of skin biopsies by classical light microscopy. Transmission-electron microscopy played a major role in establishing the cerebriform nature of the nucleus in Sézary syndrome and mycosis fungoides (MF) and enzyme histochemistry provided a means of demonstrating histiocytic or T-cell lineage.[1] However, during the last decade, immunohistochemical techniques have resulted in an explosion in the amount of scientific information available[2] including more recently, the use of DNA recombinant technology. The purpose of this review is to highlight and evaluate these advances and thereby place them in diagnostic and pathogenetic perspective for the general clinical dermatologist. It is hoped that this will also give helpful insight into developments that may be anticipated in the near future.

Immunohistochemistry

Initially, the division of cutaneous non-Hodgkins malignant lymphomas into B, T, and histiocytic types could only be achieved by the use of monoclonal antibodies on unfixed tissue.[3,4] More recently a new generation of antibodies has been highly successful in demonstrating cell lineage on fixed paraffin-embedded tissue.[5,6] Although cutaneous infiltrates displaying epidermotrophism are usually of T-cell lineage, immunophenotypic analysis is desirable for some non-epidermotrophic infiltrates; especially in the case of high-grade lesions. However, despite the undoubted value of the technique, problems can still occur. First, lineage may not be pure and dual B- and T-cell types have been reported.[7] Second, most malignant lymphoid infiltrates are accompanied by a reactive stromal lymphoid component and the latter can cause interpretive confusion. For example, a small malignant B-cell population can be masked by a larger reactive T-cell one. In addition, the latter itself may be epidermotrophic and thereby cause diagnostic problems in a superficial biopsy. Also, the cutaneous infiltrate associated with some B-cell malignancies (such as chronic lymphocytic leukaemia) may be of T-cell reactive type rather than malignant.[8]

Early enzyme histochemical techniques established that MF and Sézary syndrome were of T-cell lineage and the concept of both equating with the proposed entity of cutaneous T-cell lymphoma (CTCL) was a major advance.[9] However, advances in immunohistochemistry quickly demonstrated that other types of peripheral T-cell lymphoma existed and could present as CTCL.[10] True histiocytic lymphomas are still recognized to occur in the skin[11] but many diseases originally considered to be of histiocytic origin (such as regressing atypical histiocytosis) are, as discussed later, now known to be of T-cell lineage.

Classification of T-cell lymphomas has proved difficult but updated versions of the Kiel classification are now finding acceptance.[12,13] These classifications are based primarily on cytological criteria and post-thymic peripheral T-cell lymphomas have been divided into low- and high-grade types on the basis of being prognostically important.[14] However, with the exception of MF and Sézary syndrome the only other readily identifiable clinicopathological entity in this group is adult T-cell leukaemia/lymphoma (ATLL) associated with the human T-cell lymphotrophic leukaemia/lymphoma virus (HTLV).[15,16] The other histological T-cell lymphomas appear heterogeneous, both with regard to clinical features and immunophenotypic analysis. In addition, the Pautrier abscess, once thought to be a *sine qua non* of MF is now recognized as occurring in other T-cell lymphomas, especially ATLL.

Special mention must be made of the CD30 (Ki-1/Ber-H2) positive large-cell anaplastic lymphoma (LCAL). This belongs to the overall category of large-cell lymphoma which includes pleomorphic, centroblastic and immunoblastic types. LCAL has an identifiable histological appearance and, as initially defined, should display CD30 positivity. However, it is now known that CD30 is a lymphoid activation antigen[17] and that positivity can be seen in other lymphoid malignancies, the Reed–Sternberg cell of Hodgkins disease[17] and some reactive conditions.[18] For this reason, co-expression of epithelial-membrane antigen is a useful supplementary immunohistochemical feature. Immunophenotypically, LCAL is usually of T-cell lineage but B and 'null' types also occur.[19] LCAL occurs both as a primary cutaneous presentation and also secondary to nodal disease and cutaneous lymphoproliferative disorders such as MF and lymphomatoid papulosis.[20] Although nodal large-cell lymphomas behave as high-grade malignancies, Beljaards *et al.*[21] showed that long-term remission can be achieved in CD30-positive cases of primary T-cell cutaneous large-cell lymphoma. Similarly, Kaudewitz *et al.*[20] showed up to 6 years' remission in nine out of 11 patients with primary T-cell CD30-positive LCAL. However, a degree of caution would appear warranted as both Kaudewitz *et al.*[20] and Ralfkiaer *et al.*[18] noted rapid systemic spread in some patients. This would appear to be particularly so when LCAL has developed in the context of MF.[16,20]

It is of interest that an identical histological appearance to CD30-positive LCAL can be seen in several other cutaneous T-cell lymphoproliferative disorders including lymphomatoid papulosis,[22] regressing atypical histiocytosis,[23] and also childhood Ki-1 lymphoma.[24] Indeed the only significant difference between these and primary LCAL is that the former are characterized by the clinical feature of spontaneous regression. This indicates that the histological spectrum of LCAL must be interpreted with careful clinicopathological correlation.

Because of the diversity and uncertain prognosis of some cutaneous lymphomas, several appear as 'unclassified' or 'rare' in the updated Kiel classifications.[12,13] This includes, for example, multilobulated lymphoma and angiotrophic lymphoma (malignant angioendotheliomatosis); both of which may be of either B- or T-cell lineage.[25] Also, Jaffe's useful concept of angiocentric immunoproliferative lesions[26] is not easily included; the latter comprising a spectrum of diseases ranging from benign lymphocytic vasculitis and granulomatosis (BLAG), through lymphomatoid granulomatosis to high-grade malignant lymphoma.

As well as cell lineage, immunophenotypic analysis can also give information relating to T-cell subsets; although currently such investigations still require the use of fresh tissue. All studies have shown that malignant T-cell proliferations are most frequently of CD4 (inducer) phenotype.[1,2] Rarely, CD8 (suppressor/cytotoxic) variants of most entities including MF,[27] ATLL,[28] pagetoid reticulosis,[29] lymphomatoid granulomatosis[30] and lymphomatoid papulosis[31] have been reported. In view of the cytotoxic/suppressor functional activity of CD8 cells, several studies have attempted to assess their significance in CTCL. Divergent results have emerged with some reports describing CD8 CTCL with a good prognosis,[32] whereas others have shown a rapidly fatal outcome.[33] A possible reason for these differences has been suggested by Agnarsson *et al.*[34] who have described nine patients with CD8 CTCL and varying clinical outcomes. Agnarsson found that rapid progression was associated with the CD2-negative, CD7-positive phenotype and in comparison, chronicity associated with the CD2-positive and CD7-negative phenotype.

There has also been the interesting observation that although an excess of CD4 inducer cells is the general rule in established cases of MF, some individual cases and especially the early stages may be characterized by large numbers of CD8 cells.[35,36] From the known function of CD8 cells, it is tempting to suggest that they could be downregulating the immune response and thereby have prognostic significance. However, Vonderheid *et al.*[37] have shown that the number of CD8 cells has no significant correlation with subsequent outcome and response to treatment. In addition, a degree of caution in the area of CD4/CD8 subsets would appear warranted following a report describing a CTCL which changed from CD8 to CD4 phenotype.[38]

It has been shown that CD4 inducer T cells are heterogeneous and include suppressor (or naive) and helper (or memory) subpopulations. The suppresor inducer CD4 subset is characterized by the CD45RA (2H4) antigen which represents an epitope of the leucocyte common antigen family. A characterizing surface molecule of the helper inducer subset is the CDw29 antigen which belongs to the integrin superfamily of adhesion molecules. Also, this subset has a high density of CD45RO (UCHL1) and adhesion molecules LF-3, CD2 and LF-1. Sterry and Mielke[39] have shown that all CTCL examined (including MF, Sézary syndrome, lymphomatoid papulosis, pleomorphic and high grade T-cell lymphomas) were of helper inducer T-cell phenotype although in the peripheral blood the subsets were equally represented. This is similar to inflammatory dermatoses[40] and possibly has important pathogenetic implications. Current theories suggest that the CD4 subsets may reflect sequential stages of lymphocyte maturation before or after activation (naive and memory cells, respectively) and this could be interpreted as additional support for the theory of CTCL being associated with antigen persistence or stimulation.[41] Co-expression of CD45RA and CDw29 has been reported in a case of Sézary syndrome[42] and ATLL would appear an ideal candidate for a malignant proliferation of CDw29 cells. The cells in ATLL, although of CD4 phenotype, usually possess suppressor functional activity.[43]

The division of CD4 cells into functional subsets may have significance in the reactive stromal lymphoid population. Reinhold *et al.* have been able to isolate CD4 CD45RA suppressor inducer cells from the tumour infiltrating lymphocytes (TIL) of a patient with MF; thereby providing evidence that a subpopulation of reactive TIL may be involved in downregulating the immune response.[44]

A major expectation of monoclonal antibody technology was that the distinction between benign and malignant lymphoid infiltrates would be facilitated. Unfortunately, to date, this expectation has only been partially realized and knowledge of CD4 and CD8 subsets invariably has little bearing on the final benign or malignant diagnosis.[45] However, Abel *et al.* made the useful observation that a diagnosis of benign erythroderma was facilitated by a greater percentage of CD8 cells in the epidermis compared to the dermis.[46] While this criterion was 100% specific, it was unfortunately only 58% sensitive. Also, studies suggesting that the tumour-associated antigens (BE1 and BE2) could be useful[47] have not gained universal acceptance.[48] Despite these limitations, useful criteria for malignancy have been suggested by Picker *et al.*[49] although no single criterion has proved to be absolute in its own right and each must be interpreted with full knowledge of the clinical situation.

For both B and T cells, the criteria include monoclonality, aberrant immunophenotypic expression and lineage antigen loss. The criteria have been most critically assessed with regard to cutaneous B-cell lymphomas[50] and the most useful was found to be monoclonality. This can be demonstrated by the presence of light-chain restriction, although as some cases of cutaneous B-cell lymphomas do not express immunoglobulin[51] an absence of polytypic staining of the stromal follicular dendritic reticulum network must be used as a less satisfactory alternative. However, it should be noted that some published cases of cutaneous B-cell pseudolymphoma have been accepted as benign despite absence of dendritic cell staining.[52]

Until recently, histological techniques were not available for the demonstration of T-cell monoclonality. However, a small number of monoclonal antibodies are now available that recognize the protein specified by families of T-cell antigen receptor-variable genes.[53] Using antibodies specific for the Vβ8 and Vβ5 genes, Jack *et al.*[54] showed monoclonal proliferation in 10 out of 16 cases of plaque or tumour stage MF; although monoclonality could not be demonstrated in patch CTCL. The unexpected high frequency of one selected V gene family raises the possibility that CTCL derives from a distinct subpopulation of antigen or virus-selected T cells. Until this development, the suggestion of T-cell malignancy was best supported by an assessment of aberrant immunophenotype (such as dual CD4 and CD8 expression) or antigen loss. The commonest antigens lost in T-cell malignancies are CD4 and CD8 and also the lineage markers CD5 and CD7. It is fortuitous that the most useful lineage antigens (CD3 and βF1) are usually lost later.

It was originally thought that both MF and large plaque parapsoriasis were frequently characterized by a combined CD7 and Leu 8 negative phenotype.[55] However, subsequent work has shown that this phenotype cannot accurately distinguish between benign and malignant forms of erythroderma and that antigen loss is more constant in the later rather than early stages of MF.[56]

Antigen deficiencies must be interpreted carefully as they have been reported in benign dermatoses such as pityriasis lichenoides[57] and, in the specific case of Leu 8 deficiency, findings may relate to an expansion of the Leu 8 negative helper inducer subset rather than a true loss.

Considerable interest has been shown in the monoclonal antibody HML-1 and originally this was thought to be specific for primary intestinal T-cell lymphoma and MF.[58] However, it is now recognized that the antibody is neither specific to T cells nor to these malignancies[59] and instead may be detecting a new activation/proliferation antigen. Sperling *et al.*[58] showed that, whereas the majority of dermal cells in MF were negative for HML-1, epidermotrophic cell reactivity divided cases into those that were positive or negative. The HML-1 positive cells tended to be localized preferentially in the basal layers, whereas the negative ones were distributed throughout the epidermis or formed aggregates. Immunolabelling showed no clear correlation between HML-1 reactivity and expression of CD4 and CD8 antigens. To date it is difficult to draw definitive conclusions from these results. However, they raise the possibility that MF may not be a single entity but represents a group of heterogeneous disorders originating from different T-cell subsets, although with a similar clinical appearance.[58]

In addition to T and B cells and histiocytes, a third distinct functional population of lymphocytes are natural killer (NK) cells. Early studies using HNK-1 (Leu 7) antibodies showed no conclusive findings in cutaneous lymphoid infiltrates,[60] although occasional studies have suggested that NK cells may be the proliferating cell population in some cases of pagetoid reticulosis and lymphomatoid granulomatosis. However, as HNK-1 has a wider expression than originally recognized, these studies require to be repeated with more specific NK cell antibodies such as CD16 and CD57 (HNK-1). Although the nature of lymphokine activated killer cells remains controversial (T or NK cell origin), Wood *et al.*[61] have shown that in MF, a depressed activity in the peripheral blood may be a marker of more aggressive disease.

As well as cell lineage and subsets, immunohistochemistry can provide information relating to functional activity of lymphocytes. This includes the activation antigens CD25 (interleukin 2 receptor) and CD30 (Ki-1 or Ber H2) and the cell-cycle-associated (proliferation) nuclear antigen (Ki-67). Some lymphomas such as ATLL have a tendency to express CD25 more than MF and Sézary syndrome, but no one observation is diagnostic.

However, the results of functional antigen expression provide valuable information regarding the possible pathogenesis of cutaneous lymphoproliferative disorders. For example, Nickoloff and Griffiths[62] have shown that in MF, the predominant T cell is of memory type and that the dermal T cells, despite the variable expression of activation antigens, are Ki-67 negative and therefore in the Go resting state. In contrast, in 83% of cases of MF, the epidermal T cells are Ki-67 positive implying cycling in the G1, S, and G2-M phases. The reason for the intraepidermal cycle activation remains uncertain but presumably relates to keratinocyte–lymphocyte interaction.

In addition, activation antigen expression has been a major component of immunohistochemical evidence suggesting a close relationship between Hodgkin's disease and lymphomatoid papulosis. This has been based on the frequent expression by the large atypical cells of HLA-DR, CD25, CD30 and to a lesser extent CD15 (Leu M1).[63–65] This has resulted in Kadin's hypothesis of a common activated helper T cell origin for lymphomatoid papulosis, MF and some types of Hodgkin's disease.[66,67]

There has been considerable discussion regarding the Langerhans cell and virus hypothesis of the aetiology of MF.[1,68] The CD1 molecule is expressed on Langerhans cells and immunohistochemical evidence shows that they are an integral part of both the epidermal Pautrier abscess and lymph node in MF.[68] Recently, an important new Factor XIIIa-positive antigen presenting cell, the dermal dendrocyte, has been described[69] and its role in cutaneous lymphoproliferative disorders requires careful assessment. However, personal work to date suggests that, like the Langerhans cell, the cell has a variable distribution and state of activation within the epidermis (including Pautrier abscesses), the dermis and lymph nodes of MF (Fig. 1). Furthermore, in some cases of MF the dermal dendrocyte may be the predominant cell in the dermal infiltrate. This observation places increasing importance on the broad similarities between mucosa-associated and skin-associated lymphoid malignancies and the increasing evidence which suggests that both may be antigen stimulated and sensitive.

Another area of major current interest is cellular adhesion and its relationship to the trafficking of lymphocytes within the skin. Although cellular adhesion is extremely complex, one well-recognized association is the lymphocyte function associated antigen-1 (LFA-1) and its ligand intercellular adhesion molecule-1 (ICAM-1). Results to date in CTCL are however conflicting. Vejlsgaard *et al.*[70] found that there was a tendency in MF for keratinocytes to express less ICAM-1 as the disease progressed and that in the Sézary syndrome ICAM-1 expression was retained. This is in contrast to Nickoloff *et al.*[71] who found absence of ICAM-1 expression in the Sézary syndrome and showed that this was associated with an inability of the malignant cells to produce γ-interferon. However, observations shared by both groups were instances of epidermal HLA-DR expression in the absence of ICAM-1 expression; this is intriguing as to date no cytokine has been described which can produce this pattern.

The so-called B- and T-cell patterns of lymphoid infiltrates must have a molecular explanation in terms of homing receptors, adhesion molecules and cytokine production; as must the general tendency to lose epidermotrophism with high-grade T-cell lymphomas. It can be expected that this area will be increasingly elucidated in the near future and give valuable prognostic information regarding tumour grade and even treatment modalities.

Genotypic analysis and antigen receptor gene rearrangements

It is now recognized that there are close similarities between B- and T-cell surface-antigen receptors. On B cells, each immunoglobulin (Ig) molecule is composed of paired heavy- and light-chain proteins joined together by disulphide bonds. The T-cell receptor (TCR) is a

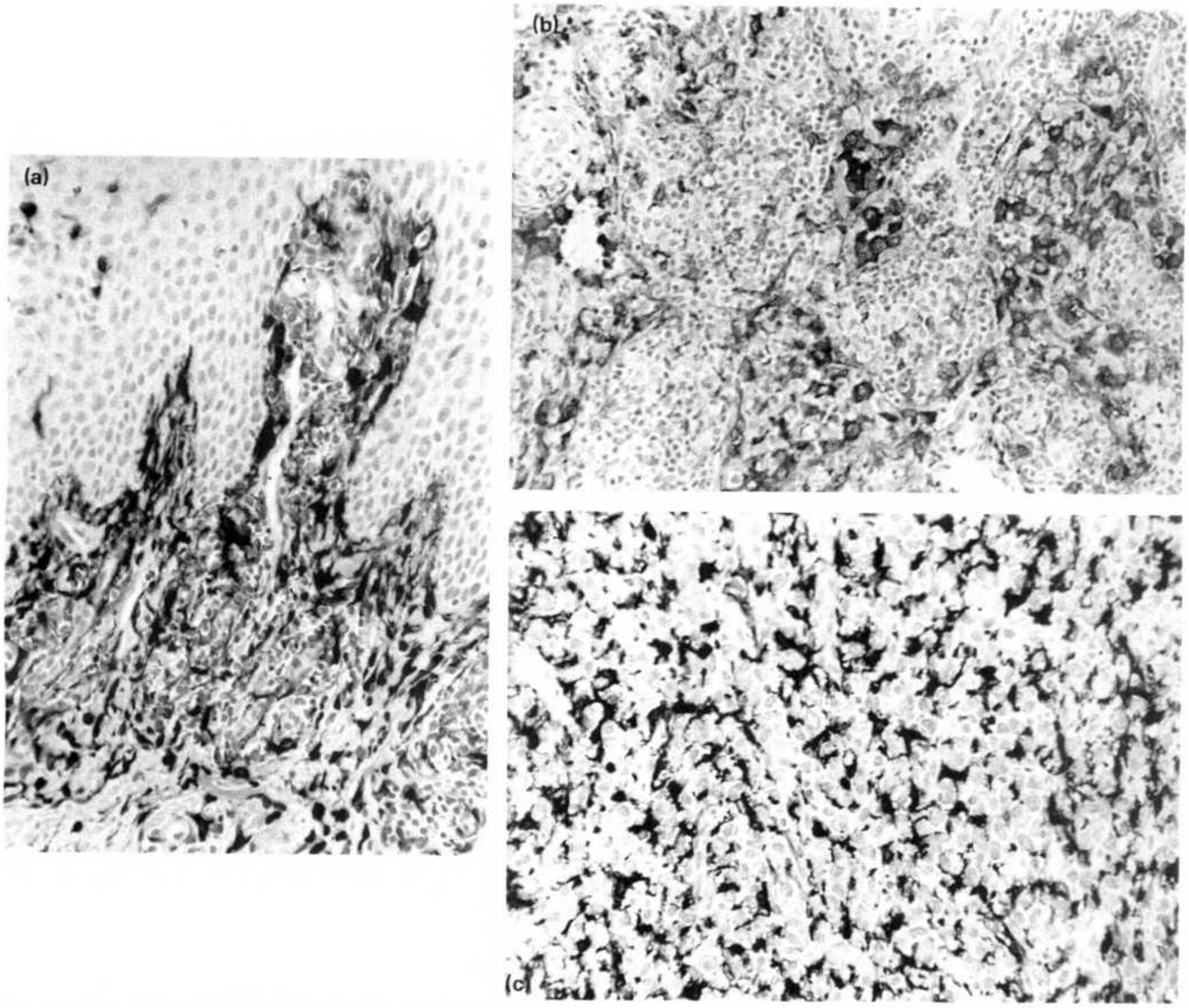

Figure 1. Factor XIIIa-positive cells (a) Skin biopsy of plaque stage mycosis fungoides (Immunoperoxidase × 400) (b) Dermatopathic lymph node in mycosis fungoides (Immunoperoxidase × 240) (c) Cutaneous nodular urticaria pigmentosa showing the close association between mast cells and XIIIa positive cells.

disulphide-linked heterodimer consisting of either single α-chain and β-protein chains or single γ- or δ-protein chains. To achieve the molecular repertoire necessary for extensive immune recognition, antigen-receptor genes must undergo somatic rearrangement of their germline configuration before a functional protein can be encoded. Antigen receptor specificity is dependent on the variable (V) region of each protein chain and rearrangement involves the selection of a V gene segment which is brought into close proximity to a joining (J) gene segment. In addition, a diversity (D) gene segment is present between the V and J segments in the case of Ig heavy chain and TCR-β-and δ-chain loci. The new contiguous sequence of V-J or V-D-J regions together with a constant (C) region, encodes the functional B- and T-cell antigen receptors. The genetic 'fingerprint' that the arrangement produces is the basis of antigen-receptor-gene arrangement studies.

Analysis of DNA rearrangement is achieved by the technique of Southern blot analysis. Chromosomal DNA is extracted from the tissue, digested with restriction endonucleases and the resulting mixture of DNA fragments separated by size using gel electrophoresis. The DNA fragments are transferred ('blotted') to a membrane and the antigen-receptor genes identified by using cloned probes prepared by recombinant DNA technology. With radioactive labelling, fragments bearing the genes appear as discreet bands on autoradiographs. The pattern of banding seen in non-lymphoid cells is known as the 'germline' configuration. In polyclonal lymphocyte proliferations, many clones contribute their own individual antigen-receptor-gene rearrangements, although none is present in sufficient quantities to be detectable as an additional band. However, a non-germline band signifies a probable monoclonal lymphocyte proliferation (Fig. 2) and the type of receptor chain rearrangement can be a useful marker of T- or B-cell lineage.

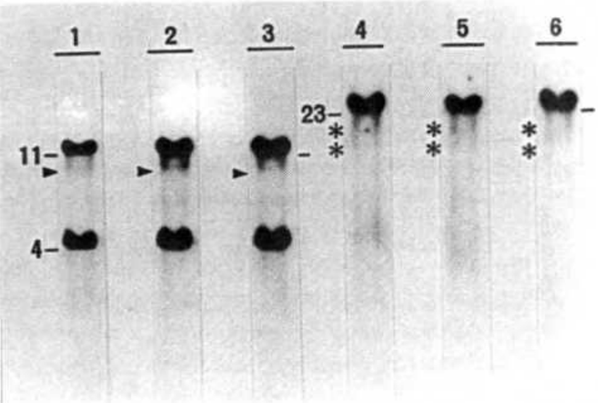

Figure 2. Autoradiographs of DNA analysis of skin nodules from a patient with suspected T-cell lymphoma. Lanes 1 and 4, nodule 1; lanes 2 and 5, nodule 2; and lanes 3 and 6, nodule 3. Lanes 1–3 digests with EcoRI and lanes 4–6 digests with Bam H1. Dashes indicate unrearranged germ-line fragments, arrows and asterisks indicate the identically rearranged TCR beta genes. Numbers indicate mol. wt. in kb. (From: Ideyama S, Taraka T, Akagi M *et al. Br J Dermatol* 1988; **118:** 264.)

T-cell lymphoproliferative disorders

Studies in which the reported TCR β-gene rearrangement can be correlated with the clinical stage of MF or Sézary syndrome are summarized in Table 1. A reasonably predictive pattern emerges in that β-gene rearrangement occurs frequently in the peripheral blood in Sézary syndrome, in the skin in tumour stage MF and in the lymph nodes showing histological involvement by Sézary syndrome or MF. However, β-gene rearrangement has rarely been demonstrated in patch-stage MF and is often not detectable in plaque stage. There are various explanations for an absence of identifiable gene rearrangement including chromosome loss, co-migration of rearranged and germline bands and alternative antigen-receptor rearrangements. However, these possibilities do not appear particularly relevant to MF and a lack of rearrangement is generally interpreted to indicate that either the early stage of MF is polyclonal or that any monoclonal cell population present is below the sensitivity of Southern blot analysis (usually regarded as 1–5% of the total cell population). An interesting finding has been the presence of β-gene rearrangement in approximately 75% of lymph nodes showing dermatopathic change on light microscopy.[72] This is in agreement with previous studies showing that extracutaneous spread is extremely common in MF[80] and provides a reason why palpable lymphadenopathy in MF is an unfavourable prognostic sign irrespective as to whether histological mycotic involvement is present.[81] It also supports the view that nodal sites are important in the pathogenesis of mycosis fungoides and Sézary syndrome[82–84] and

Table 1. Beta T-cell receptor rearrangements in mycosis fungoides and Sézary syndrome

	Mycosis fungoides						
	Skin		Lymph node		Blood		Sézary syndrome/erythrodermic mycosis fungoides
	Stage I/II	Stage III/IV	Stage IVa	Stage IVb	Stage I/II	Stage III/IV	Blood and/or lymph node
Weiss *et al.*[72]	—	5 (5)	7 (9)	8 (8)	—	2 (2)	—
Weiss *et al.*[73]	—	—	—	—	2 (13)	1 (4)	3 (3)
Bertness *et al.*[74]	—	—	0 (1)	7 (7)	—	—	11 (11)
Waldmann *et al.*[75]	—	—	—	—	—	—	5 (5)
O'Connor[76] and Ralfkiaer *et al.*[77]	0 (12)	10 (10)	2 (2)	—	—	—	5 (5)
Bignon *et al.*[78]	1 (1)	—	—	—	—	—	—
Peters *et al.*[79]	1 (1)	—	—	0 (1)	—	—	—

Results are shown as number of cases with TCR β-gene rearrangement. Figures in parenthesis are the total number of cases studied. Staging key (Scandinavian MF Cooperative Group): Stage 1, patch stage; Stage 2, plaque stage; Stage 3, tumour (nodule) stage; Stage IVa, dermatopathic lymphadenopathy; Stage IVb, lymphadenopathy with mycotic involvement.

gives insight as to why purely cutaneous treatment modalities are often not curative.

Some studies have demonstrated rearrangement of TCR γ[73,85] and α[86] genes and this can be useful when β-gene rearrangement is absent.[85] Care has to be taken, however, in the interpretation of TCR γ non-germline bands in the absence of β-gene rearrangements. TCR γ rearrangement is much more limited in the V segment compared to the β chain and non-germline bands may represent the presence of several clones having the same rearrangement rather than a monoclonal proliferation.

The detection of non-germline rearranged bands in the more advanced stages of MF and Sézary syndrome indicates that both are clonal T-cell lymphoproliferative disorders. In addition, the presence of one or two identical rearranged genes from different sites within the same patient[72] suggests that the same clone is involved and has been derived from a transformation event within a single T cell. In the clinical context, treatment and prognostic assessment are totally dependent on disease staging. Accordingly, it is important that Weiss *et al.*[72] have convincingly shown that TCR gene analysis can significantly improve staging accuracy and also be used to assess whether occult disease is present during clinical remission.[73]

TCR gene analysis has also thrown doubt on the validity of assessing peripheral blood involvement in MF and Sézary syndrome by light microscopic examination for Sézary cells. In a series of 26 patients, Weiss *et al.*[73] found peripheral blood TCR rearrangement in one case with no Sézary cells and, in comparison, a germline configuration in two cases with 3–4% of circulating Sézary-like cells. Also, Bendelac *et al.*[87] failed to find TCR β-gene rearrangement in a patient with 67% circulating Sézary-like cells, although rearrangement was present in a skin biopsy. Non-neoplastic circulating Sézary-like cells have been reported in a variety of benign dermatoses and it would appear that similar reactive cells can occur in the peripheral blood in MF and Sézary syndrome.

Unlike most lymphomas, CTCL often contains a polymorphous infiltrate comprised not only of malignant T cells but also benign lymphocytes and other inflammatory cells. Ho *et al.*[88] have cultured and cloned T cells from the lesions of CTCL and analysed the TCR β- and γ-gene rearrangements of the cloned lymphocytes. None of the cloned cells exhibited the same gene rearrangements as the predominant, presumed malignant, clone found in the peripheral blood and skin, even though the malignant clones were present in the original *in vitro* lymphocyte culture. Twelve different T-cell clones were derived from each patient suggesting that they represented the presumably reactive T cells in the lesions. The ability to selectively grow these reactive tumour-infiltrating lymphocytes could be used in adaptive immunotherapy.

Lymphomatoid papulosis has been the subject of several genotypic studies. Weiss *et al.*[89] studied six patients and found TCR β-gene rearrangement in five and γ-gene rearrangement in four. However, in three of these patients there was coexisting MF or lymphoma. Classical lymphomatoid papulosis has been analysed by Kadin *et al.*[90] in five patients and β-gene rearrangements were identified in four. Interestingly, subsequent lymphoma was only associated with one of the clonal cases. Kaudewitz *et al.*[91] have reported a patient with lymphomatoid papulosis who subsequently developed a nodal LCAL. Identical β-gene rearrangements were found in the skin and lymph node and the authors suggested that a T-cell clone with metastatic potential had developed within a cutaneous lesion. Although immunoglobulin gene analysis was not undertaken by either Weiss or Kadin, rearrangements were not detected by Whittaker *et al.*[92] Both Weiss and Kadin found no correlation between Willhemze histological type and the presence or absence of TCR gene rearrangement. However, in contrast, Whittaker *et al.*[92] found TCR rearrangement indicative of type B (characterized by large cerebriform cells) rather than type A (characterized by Reed–Sternberg-like cells). In patients without associated malignancy, Weiss *et al.*[89] found between one and three β- and γ-gene rearrangements. Explanations for more than two rearrangements of TCR genes could include naturally occurring DNA polymorphism of the germline configuration, extra chromosomes or chromosomal breakage within the sequence hybridized by the probe. However, the most likely explanation in lymphomatoid papulosis is a multiclonal origin of the disease. In addition, although not confirmed by Kadin, Weiss showed varying patterns of the gene rearrangements in three specimens from different sites in the same patients. This suggested that different lesions could contain different T-cell clones. The presence of multiclonal and multifocal lesions in lymphomatoid papulosis may have important pathogenetic implications in view of similar multiclonal B-cell tumours that have been demonstrated in immunodeficient patients infected with Epstein–Barr virus.[93] This comparison raises the possibility of a role for either some form of viral infection and/or immunological deficiency in lymphomatoid papulosis. It is conceivable that the lesions of lymphomatoid papulosis develop when T-cell expansion is greater than the host immune

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response and regress when the balance alters in favour of the latter. The development of malignant lymphoma could theoretically supervene following multistep molecular events that progress from polyclonal to clonal proliferations and overwhelm host immune mechanisms.

B-cell lymphoproliferative disorders

The value of immunoglobulin gene rearrangements as markers of clonality has been recognized for some time and in general all cases of B-cell non-Hodgkin's lymphoma show varying combinations of heavy-and light-chain rearrangement.[94] It is perhaps for this reason that cutaneous B-cell lymphomas have been poorly studied. However, in one report, Berti *et al.* analysed 13 cases and detected one or two rearrangements for immunoglobulin heavy chain in 11 cases and for kappa or lambda light chain in all cases.[95]

A valuable application of the technique has been in the area of cutaneous B-cell lymphoid hyperplasia. Weiss *et al.* initially studied two cases which showed no immunoglobulin gene rearrangement.[96] However, in a heterogeneous series of 18 cutaneous pseudolymphomas, Wechsler *et al.*[97] identified one patient with cutaneous lymphoid hyperplasia and immunoglobulin gene rearrangement, despite the lesion having a polyclonal appearance on immunohistochemistry. Wood *et al.*[98] have analysed 14 patients with the clinical, histopathological and polyclonal immunohistochemical findings of cutaneous lymphoid hyperplasia. Five patients showed heavy-chain gene rearrangements and of these three possessed additional light-chain gene rearrangements. One patient exhibited clinical resolution of the clonal lesions without therapy and one experienced progression into a large B-cell lymphoma over a 2-year period of follow-up. The Ig gene rearrangements of this lymphoma were identical to those of the prior lesion. Results similar to these have been reported in both ocular and salivary gland lymphoid infiltrates, and it is possible that occult clonal B-cell populations may be not unusual among extranodal lymphoid hyperplasias containing B cells that are polyclonal by immunohistological criteria. Further studies are required to assess whether the presence of B-cell monoclones within the lesions of cutaneous lymphoid hyperplasia defines a subset with a high risk of developing subsequent overt cutaneous B-cell lymphoma. However, in the interim, it would appear wise for patients with genotypically clonal cutaneous lymphoid hyperplasia to be followed up carefully.

The presence of multiple Ig rearrangements in B cells poses more problems with interpretation then multiclonal TCR rearrangements. The Ig gene remains susceptible to somatic mutations even after rearrangement and the diagnosis of mono- or multi-clonality must depend on additional genetic analysis.

Cell lineage identification

Until recently, the cell lineage of several dermatopathological diseases has remained obscure. As with lymphomatoid papulosis, immunohistochemical findings have frequently been diverse with often no definitive answer emerging.

Regressing atypical histiocytosis (RAH) was first defined by Flynn *et al.*[99] as a proliferation of atypical 'histiocytes' that formed nodulo-ulcerative lesions in skin. However, there is now substantial evidence that many neoplasms judged to be histiocytic in derivation based on morphological criteria are, in fact, of T-cell origin.[100] Therefore, although very few cases have been analysed, it is of importance that Headington *et al.*[23] found rearrangements of the TCR β gene and partial deletion of the γ gene in a classical case of RAH. This is highly suggestive of a monoclonal proliferation of T-cell origin. RAH has features in common with lymphomatoid papulosis including histological features of malignancy, a tendency for lesions to undergo spontaneous remission and a risk of development of systemic lymphoma. It is entirely possible that the pathogenetic features discussed with lymphomatoid papulosis are equally applicable to RAH and indeed to other cutaneous T-cell lymphoproliferative disorders with similar characteristics. Kadin *et al.*[24] have described a clinicopathological syndrome among children and adolescents of a Ki-1 lymphoma characterized clinically by frequently regressing skin lesions and peripheral lymphadenopathy. Comparison has been made between this syndrome and RAH, and TCR β-gene rearrangements were found in the one case analysed to date.

In 1972, Liebow *et al.*[101] described a new entity entitled lymphomatoid granulomatosis. This was characterized by an angiocentric and angiodestructive atypical lymphohistiocytic and granulomatous infiltrate. Although the disease primarily affects the lungs, cutaneous involvement is extremely common and may be the initial presentation. The clinical course is extremely variable and some cases may evolve into an overt systemic lymphoma. This has resulted in uncertainty in its nosological position and both 'inflammatory' and 'malignant' origins have been suggested. To date, two cases have been reported with genotypic analysis[30,102] and both have possessed rearrangement of the TCR β gene. Both cases were unusual, however, in that they were associated with other malignancies (lethal midline granuloma and myeloblastic leukaemia).

Pagetoid reticulosis is a rare disorder comprising either solitary or localized cutaneous plaques (Woringer–Kolopp disease) or a disseminated form (Ketron–Goodman variant). The nature of the intraepidermal cells has been controversial and conflicting evidence has suggested a T-cell, monocyte, histiocyte or even Merkel-cell origin. Similarly, there has been disagreement about whether the disease is a reactive or neoplastic disease. Wood *et al.*[103] have undertaken genotypic analysis on one case of Woringer–Kolopp disease and found clonal rearrangements of both TCR β- and γ-chain genes. This is supportive of a T-cell origin and confirms clinical suspicions of a close relationship with CTCL.

Granulomatous slack disease is a perplexing disorder characterized by lax bulky folds of skin and a dense granulomatous cellular infiltrate. In addition, unusual histological features include lymphocyte epidermotrophism similar to CTCL and a clinical association with Hodgkin's disease in some patients. A clonal nature of the disease has been shown by LeBoit *et al.*[104] who have demonstrated TCR β-gene rearrangement in three cases.

It is only recently that so-called malignant angioendotheliomatosis has been shown to be an angiotropic lymphoma by immunophenotypic analysis. In addition, genotypic analysis has confirmed the previous immunohistochemical findings that the cells may be of either B- or T-cell origin.[105,106] However, one of the cases[105] showed both TCR β gene and Ig heavy- and light-chain-gene rearrangements. An explanation for this is the presence of two separate clonal populations; one with Ig and the other with TCR rearrangement. However, other possible explanations include karyotype abnormalities, recombinase effects or a transformational event in a pluripotential stem cell not committed to the B or T cell lineage. Dual genotypes involving IgG heavy-chain gene and TCR β-gene rearrangements have been identified by Weiss *et al.*[72] and Ralfkiaer *et al.*[77] in CTCL. A similar genotype has also been confirmed by Berger *et al.*[107] in the erythrodermic skin lesions of four out of 13 patients with leukaemic cutaneous T-cell lymphoma.

These findings show that antigen receptor analysis can be useful in assessing cell lineage. However, uncertainties can occur which indicate that it is not absolute in all cases and must be assessed with other information, such as immunophenotypic analysis.

Clonality and malignancy

An important issue raised by antigen receptor analysis is the relationship between clonality and malignancy. As monoclonality has become regarded as a *sine qua non* of malignancy, it is tempting to diagnose malignancy in lymphoproliferative disorders showing monoclonality. It is clear, however, that not all diseases with a monoclonal population are clinically progressive and, in reverse, proof of clonality can be elusive in some lymphomas.[108] Benign monoclonal B-cell gammopathy when followed for long periods of time seldom progresses to myeloma. Also, clonal T-cell rearrangements have been found in over 50% of cases of T-γ-lymphoproliferative disease,[109] although in these patients overt malignancies have not been reported to develop. In addition, Weiss *et al.*[96] identified TCR β-gene rearrangements from three cases of pityriasis lichenoides et acuta (Mucha–Habermann disease), including the demonstration of similar rearrangements at different sites. There are several non-dermatological settings where antigen receptor rearrangements can be encountered and a lymphoid monoclone can sometimes but not always develop into malignant lymphoma.[110] These include post-transplant immunosuppression, congenital immunodeficiencies, acquired immunodeficiency syndrome and angioimmunoblastic lymphadenopathy. Interestingly, both malignant B-cell lymphomas and EBV mitogenically stimulated B cells can display Ig rearrangements indicative of clonal expansion. However, removal of immunosuppressive therapy can also reverse the clonal growth. Patients on immunosuppressive therapy after organ transplantation are at an increased risk of developing Epstein–Barr virus (EBV)-associated B-cell lymphoproliferative disorders, some evolving into malignant lymphomas and others not. Unfortunately, to date, detailed molecular investigations into the possible role of EBV and cutaneous lymphoproliferative disorders such as lymphomatoid papulosis and PLEVA are not available. However, rising titres to EBV have been reported in lymphomatoid papulosis.[111]

Mielka *et al.*[112] have suggested that the term 'benign cutaneous lymphoma' should be applied to conditions such as pagetoid reticulosis which display TCR rearrangements but are characterized by a benign biological course. This proposal is worthy of serious consideration as the strict definition of lymphoma is a neoplastic proliferation of lymphoid cells. It is only by common, and perhaps lapse, usage that the prefix malignant is now automatically implied. It is entirely conceivable that some stages of other clonal cutaneous T-cell lymphopro-

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liferative disorders could be placed under this heading; as could some cases of clonal B-cell proliferation manifesting as lymphoid hyperplasia. In the final instance, however, it would appear likely that many cutaneous lymphoproliferative conditions could lie on a continuous spectrum of polyclonal and clonal reactive disorders, benign clonal lymphoma and finally malignant lymphoma. This is analogous to the ulcerative colitis—carcinoma spectrum in the bowel and in the skin it is proposed that, after the initial cutaneous inflammatory stage, lymphoma evolves in a multistep sequence by a series of molecular and genetic events awaiting elucidation.[113]

TCR gene rearrangements may prove to be useful in the study of cutaneous inflammatory disorders. Just as dominant idiotypes of immunoglobulins are present in diseases such as rheumatoid disease, so dominant T-cell lines may play a role in inflammatory skin disorders. It is relevant, although in culture, that T cells from rheumatoid synovium have shown TCR β-gene rearrangements.[114] To date, however, with the exception of PLEVA, most studies have failed to show TCR gene rearrangements in cutaneous inflammatory disorders. An exception has been Bignon *et al.*[115] who reported TCR β-gene rearrangement in six out of seven T-cell pseudolymphomas including Jessner's lymphocytic infiltrate, actinic dermatitis, drug eruption and Lyme disease. In contrast, Griesser *et al.* found the presence of clonal rearrangements was a useful predictor of later frank malignancy in cutaneous T-cell rich pseudolymphomas.[116] In addition, it must be emphasized that polyclonal proliferation can behave aggressively, as shown by Eckert *et al.*[117] in their series of polyclonal follicular pseudolymphomas.

Sufficient evidence is already available to seriously question whether the term lymphoma is justifiable for the early stages of mycosis fungoides, when clonal proliferation of T cells can rarely be demonstrated. Also, it is hoped that increased molecular information will gradually obviate the necessity to use the term 'pseudolymphoma' as a pathological diagnosis. Undoubtedly, some dermatologists may continue to defend the use of the term pseudolymphoma on the basis of being a clinically useful concept and that the histological appearance may mimic malignant lymphoma. However, in the new molecular world, usage of the term has increasingly little scientific justification. The encompassing term 'clonal dermatoses' has recently been suggested for this new group of inflammatory and lymphomatous cutaneous disorders.[113]

Molecular cytogenetics

Many malignancies are associated with chromosomal rearrangements and in lymphoid neoplasia, chromosomal translocation can relate to breakages in or near antigen-receptor genes. This induces the transcription of genes (cellular or proto-oncogenes) whose products have a major role in growth and the pathogenesis of neoplasia. For example, non-Hodgkin's follicular lymphoma is frequently characterized by a non-random t (14:18) chromosomal translocation[118] which results in the juxtaposition of a proto-oncogene, termed bcl-2 (B-cell leukaemia-lymphoma 2), on chromosome 18 with the Ig heavy-chain locus on chromosome 14. It is apparent that bcl-2 encodes a protein that is involved in follicular proliferation. The availability of DNA probes specific for bcl-2 and its flanking chromosomal segments now permits a molecular cytogenetic analysis of this feature.

To date, there have been few molecular cytogenetic studies of cutaneous lymphoproliferative disorders. However, Wood *et al.*[98] have used PFL-1, 2 and 3 probes to analyse possible t (14:18) translocation in 14 patients with cutaneous B-cell lymphoid proliferations. Although five of their patients showed Ig light-and/or heavy-chain rearrangements, none showed t (14:18) translocation. As some nodal follicular lymphomas do not display the t (14:18) translocation, it remains difficult to draw conclusions regarding the origin of cutaneous B-cell lymphomas. However, its absence raises the question as to whether some are of non-germinal centre origin and arise from other areas such as the mantel zone. Results of bcl-2 and bcl-1 analysis in cutaneous B-cell lymphoproliferative disorders are awaited with interest.

Clonal chromosomal abnormalities are well described in cutaneous T-cell lymphoma but most appear random. No constant abnormalities have yet been described involving chromosome 7,[119,120] which carries the TCR β gene. Also, preliminary studies of T-cell neoplasia have not shown any detectable structural alterations of chromosome 18 in the vicinity of bcl-2.[118]

However, cellular oncogenes can be activated in alternative ways to chromosomal rearrangements including point mutations, gene amplification and gene expression. Also, the area of tumour suppressor gene/anti-oncogene deletion remains inadequately explored in cutaneous lymphomas. There can be little doubt that molecular technology is about to achieve a major breakthrough and elucidate cytogenetic abnormalities that will be used in the diagnosis, classification, staging and treatment of cutaneous lymphoma.

Molecular virus integration and lymphoproliferative disorders

Adult T cell leukaemia/lymphoma (ATLL) was the first human T cell malignancy to be definitively associated with a viral aetiology (HTLV) and classical cases are easily diagnosed by serological tests. However, there are several reports of atypical cutaneous cases being diagnosed by the demonstration of proviral DNA integration by Southern blot analysis.[121,122] In addition, identical restriction patterns in digests provide an alternative method of demonstrating clonal proliferations associated with virus infection.[122,123] This has important pathogenetic implications as the finding implies virus integration prior to clonal proliferation.

It has been suggested that retroviruses may have a pathogenetic role in MF and Sézary syndrome. Wantzin *et al.* found HTLV antibodies in 10 of 68 Danish patients with cutaneous T-cell lymphoma[124] and Ranki *et al.*[125] have also found cross-reacting antibodies to GAG proteins of HTLV-1 and HTLV-3 in patients with MF and the closely associated condition, large plaque parapsoriasis. The role of HTLV-1 has been further supported by the identification of clonally integrated proviral DNA in a case of MF with lymphadenopathy.[126]

The possible role of herpes viruses in the pathogenesis of CTCL has been poorly investigated. However, Lee *et al.*[127] reported that the skin of patients with MF contained the herpes simplex virus genome and the new human B-lymphotrophic virus (human herpes virus-6) has been isolated from a patient with an MF-like illness.[128] Until recently, EBV DNA has only been found in B lymphocytes. However, Jones *et al.*[129] have demonstrated the presence of clonally integrated EBV DNA in three patients with T-cell lymphomas, one of which was characterized by a generalized erythematous skin eruption and systemic spread. In addition, clonally integrated EBV DNA has been found in a substantial percentage of cases of Hodgkin's disease.[130,131] In view of the proposed relationship between Hodgkin's disease, lymphomatoid papulosis and MF,[67] urgent molecular viral studies are required in these diseases. A prediction that EBV could have a major role in the pathogenesis appears extremely reasonable. This proposal is also supported by the observation that EBV can cause CD25 and CD30 activation-antigen expression and as already discussed, there are characteristic immunohistochemical findings in this group of disorders. Also, using immunoblot analysis, Lee *et al.*[132] have found Epstein–Barr-associated nuclear antigen (EBNA) antibodies in all 21 patients with plaque/tumour stage MF compared to only 12 control psoriatic patients.

It is now generally accepted that lethal midline granuloma and lymphomatoid granulomatosis are similar angiocentric T-cell lymphomas. Accordingly, the study by Harabuchi *et al.*[133] into EBV and nasal T-cell lymphomas requires repeating with other cutaneous angiocentric T-cell lymphomas. Harabuchi found that although the EBV receptor antigen was absent, all cases tended to express the EBNA2 gene and late membrane protein gene, both of which are believed to be responsible for the oncogenic function of EBV.

The future and conclusions

The technique which, more than any other, will influence the immediate future of dermatopathology is the polymerase chain reaction (PCR).[134] Using PCR, a minute amount (1 μg) of a segment of DNA or RNA (after converting to cDNA via reverse transcriptase) can be amplified more than a millionfold in only a few hours using an automated procedure. Significantly, the technique can be used on formalin-fixed paraffin-embedded tissue and this will permit a fascinating retrospective analysis of archival dermatopathological material.

There is evidence that PCR will be applied for detecting B- and T-cell clonality[135,136] and it has already been used to identify proviral HTLV-1 integration during both the indolent cutaneous and lymphomatous stage of HTLV-1 infection.[137] A rapid increase in information regarding retrovirus and herpes virus infection in cutaneous lymphoma must be envisaged. The ability to amplify chromosomal translocations, such as t (14:18), is enabling sensitive diagnostic tests to be developed and PCR with allele specific oligonucleotide (ASO) hybridization can demonstrate point mutations, some of which may be associated with oncogene activation.

It should not be forgotten that the skin is heterogeneous with regard to cells and the localization of DNA and RNA sequences to specific cells remains essential. This can be achieved by *in situ* hybridization using either radioactive or non-radioactive probes and important areas will include the intracellular localization of viral nucleic acids, cytokine messenger RNA and immunoglobulin RNA for clonality.[138]

Finally, some clinical dermatologists reading this review could be disheartened, especially if they have no access to these new techniques. They can be reassured, however, that during the coming period of molecular dermatopathology data collection, classical diagnostic gold standards must remain unaltered: namely a combined clinicopathological diagnosis based on clinical features and interpretation of the skin biopsy by light microscopy. At the moment, the results from these new investigative techniques should be regarded as a means of modulating and advancing current views and concepts. We must accept, however, that the era of the molecular dermatopathologist is already here and that by the year 2000 molecular dermatopathological diagnosis and classifications will be standard usage.

References

1 Slater DN. Lymphoproliferative conditions of skin. In: *Recent Advances in Histopathology* (Anthony PP, MacSween NM, eds), Vol 12. Edinburgh: Churchill Livingstone, 1984; 83–110.

2 Slater DN. Recent developments in cutaneous lymphoproliferative disorders. *J Pathol* 1987; **153**: 5–19.

3 Wood GS, Burke JS, Horning S *et al.* The immunologic and clinicopathologic heterogeneity of cutaneous lymphomas other than mycosis fungoides. *Blood* 1983; **62**: 464–72.

4 Ralfkiaer E, Saati T, Bosq J *et al.* Immunocytochemical characterisation of cutaneous lymphomas other than mycosis fungoides. *J Clin Pathol* 1986; **39**: 553–63.

5 Faure P, Chittal S, Gorguet B *et al.* Immunohistochemical profile of cutaneous B-cell lymphoma on cryostat and paraffin sections. *Am J Dermatopath* 1990; **12**: 122–33.

6 Cerrani L, Smolle J, Soyer P *et al.* Immunophenotyping of cutaneous lymphoid infiltrates in frozen and paraffin-embedded tissue sections: a comparative study. *J Am Acad Dermatol* 1990; **22**: 405–12.

7 Dreno B, Stalder JF, Bureau B *et al.* Cutaneous malignant lymphoma with dual B and T cell markers. *Lancet* 1984; **ii**: 459.

8 Greenwood R, Barker DJ, Tring FC *et al.* Clinical and immunohistological characterisation of cutaneous lesions in chronic lymphocytic leukaemia. *Br J Dermatol* 1985; **113**: 447–53.

9 Edelson RL. Cutaneous T cell lymphoma. *J Am Acad Dermatol* 1980; **2**: 89–106.

10 Nasu K, Said J, Vonderheid E *et al.* Immunopathology of cutaneous T-cell lymphomas. *Am J Pathol* 1985; **119**: 436–47.

11 Ralfkiaer E, Delsol G, O'Connor NT *et al.* Malignant lymphomas of true histiocytic origin. A clinical, histological, immunophenotypic and genotypic study. *J Pathol* 1990; **160**: 9–17.

12 Suchi T, Lennert K, Tu L-Y *et al.* Histopathology and immunohistochemistry of peripheral T-cell lymphomas: a proposal for their classification. *J Clin Pathol* 1987; **40**: 995–1015.

13 Stansfield AG, Diebold J, Kapanci Y *et al.* Updated Kiel classification of non-Hodgkin's lymphomas. *Lancet* 1988; **i**: 292–3.

14 Krajewski AS, Myskow MW, Cachia PG *et al.* T-cell lymphoma morphology, immunophenotype and clinical features. *Histopathology* 1988; **13**: 19–41.

15 Maeda K, Takahashi M. Characterisation of skin infiltrating cells in adult T-cell leukaemia/lymphoma (ATLL): clinical, histological and immunohistochemical studies on eight cases. *Br J Dermatol* 1989; **121**: 603–12.

16 Sterry W, Korte B, Schubert C. Pleomorphic T-cell lymphomas and large cell anaplastic lymphoma of the skin. *Am J Dermatopathol* 1989; **11**: 112–23.

17 Stein H, Mason DY, Gerdes J *et al.* The expression of the Hodgkin's disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: evidence that Reed–Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. *Blood* 1985; **66**: 848–58.

18 Ralfkiaer E, Bosq J, Gatter KC *et al.* Expression of a Hodgkin and Reed–Sternberg cell antigen (Ki-1) in cutaneous lymphoid infiltrates. *Arch Dermatol Res* 1987; **279**: 285–92.

19 Feller AC, Sterry W. Large cell anaplastic lymphoma of the skin. *Br J Dermatol* 1989; **121**: 593–602.

20 Kaudewitz P, Stein H, Dallenbach F *et al.* Primary and secondary cutaneous Ki-1 (CD30) positive anaplastic large cell lymphomas. *Am J Pathol* 1989; **135**: 359–67.

21 Beljaards RD, Meijer CJLM, Scheffer E *et al.* Prognostic significance of CD30 (Ki-1/Ber-H2) expression in primary cutaneous large cell lymphoma of T origin. *Am J Pathol* 1989; **135**: 1169–78.

22 Ralfkiaer E, Stein H, Wantzin GL *et al.* Lymphomatoid papulosis. Characterisation of skin infiltrates by monoclonal antibodies. *Am J Clin Pathol* 1985; **84**: 587–93.

23 Headington JT, Roth MS, Ginsburg D *et al.* T cell receptor gene rearrangement in regressing atypical histiocytosis. *Arch Dermatol* 1987; **123**: 1183–7.

24 Kadin ME, Sako D, Berliner N *et al.* Childhood Ki-1 lymphoma presenting with skin lesions and peripheral lymphadenopathy. *Blood* 1986; **68**: 1042–9.

25 Sheibani K, Battifora H, Winberg CD *et al.* Further evidence that 'malignant angioendotheliomatosis' is an angiotropic large-cell lymphoma. *New Engl J Med* 1986; **314**: 943–8.

26 Jaffe ES. *Surgical Pathology of the Lymph Nodes and Related Organs.* Philadelphia: WB Saunders, 1985; 241–6.

27 Ohkohchi K, Aiba S, Tagami H. OKT8—reactive cell mycosis fungoides. *Arch Dermatol* 1986; **122**: 20–2.

28 Doi S, Nasu K, Arita Y *et al.* Immunohistochemical analysis of peripheral T-cell lymphoma in Japanese patients. *Am J Clin Pathol* 1989; **91**: 152–8.

29 MacKie RM, Turbitt ML. A case of pagetoid reticulosis bearing the T cytotoxic suppressor surface marker on the lymphoid infiltrate: further evidence that pagetoid reticulosis is not a variant of mycosis fungoides. *Br J Dermatol* 1984; **110**: 89–94.

30 Whittaker S, Foroni L, Luzzatto L *et al.* Lymphomatoid granulomatosis—evidence of a clonal T-cell origin and an association with lethal midline granuloma. *Q J Med* 1988; **68**: 645–55.

31 Kadin ME. Characteristic immunologic profile of large atypical cells in lymphomatoid papulosis. Possible implications for histogenesis and relationship to other diseases. *Arch Dermatol* 1986; **122**: 1388–90.

32 Caputo R, Monti M, Berti E, Cavicchini S. A verrucoid epidermotrophic OKT-8 positive lymphoma. *Am J Dermatopathol* 1983; **5**: 159–64.

33 Fujiwara Y, Abe Y, Kuyama M *et al.* CD8 cutaneous T-cell lymphoma with Pagetoid epidermotrophism and angiocentric and angiodestructive infiltration. *Arch Dermatol* 1990; **126**: 801–4.

34 Agnarsson BA, Vonderheid EC, Kadin ME. Cutaneous T cell lymphoma with suppressor/cytotoxic (CD8) phenotype. Identification of rapidly progressive and chronic subtypes. *J Am Acad Dermatol* 1990; **22**: 569–77.

35 Tosca AD, Varelzidis AG, Economidou T *et al.* Mycosis fungoides; evaluation of immunohistochemical criteria for the early diagnosis of the disease and differentiation between stages. *J Am Acad Dermatol* 1986; **15**: 237–45.

36 Tan RS-H, Macleod TIF, Dean SG. Pagetoid reticulosis, epidermotrophic mycosis fungoides and mycosis fungoides: a disease spectrum. *Br J Dermatol* 1987; **116**: 67–77.

37 Vonderheid EC, Tan E, Sobel EL *et al.* Clinical implications of immunologic phenotyping in cutaneous T cell lymphoma. *J Am Acad Dermatol* 1987; **17:** 40–52.

38 Weiss LM, Crabtree GS, Rouse RV, Warnke RA. Morphologic and immunologic characterisation of 50 peripheral T cell lymphomas. *Am J Pathol* 1985; **118:** 316–324.

39 Sterry W, Mielke V. CD4 cutaneous T cell lymphomas show the phenotype of helper/inducer T cells (CD45RA negative, CDw29 positive). *J Invest Dermatol* 1989; **93:** 413–16.

40 Bos JD, Hagenaars C, Das PK *et al.* Predominance of 'memory' T cells (CD4, CDw29) over 'naive' cells (CD4, CD45RA) in both normal and diseased skin. *Arch Dermatol Res* 1989; **281:** 24–30.

41 Tan R S-H, Butterworth CM, McLaughlin H *et al.* Mycosis fungoides—a disease of antigen persistence. *Br J Dermatol* 1974; **91:** 607–16.

42 Mackenzie RH, Milton JI, Sewell FH. Coexpression of 2H4 (suppressor-inducer) and 4B4 (helper-inducer) antigens on neoplastic CD4 T cells. *J Pathol* 1989; **158:** 339A.

43 Waldmann TA, Greene WC, Sarin PS *et al.* Functional and phenotypic comparison of human T cell leukaemic/lymphoma virus positive adult T cells with human T cell leukaemic/lymphoma negative Sezary leukaemia and their distinction using anti-Tac. *J Clin Invest* 1984; **73:** 1711–18.

44 Reinhold U, Pawelec G, Fratila A *et al.* Phenotypic and functional characterisation of tumour infiltrating lymphocytes in mycosis fungoides: continuous growth of CD4 CD45R T cell clones with suppressor inducer activity. *J Invest Dermatol* 1990; **94:** 304–9.

45 Piepkorn M, Marty J, Kjeldsberg CR. T cell subset heterogeneity in a series of patients with mycosis fungoides and Sezary syndrome. *J Am Acad Dermatol* 1984; **11:** 427–32.

46 Abel EA, Lindae ML, Hoppe RT, Wood GS. Benign and malignant forms of erythroderma: cutaneous immunophenotypic characteristics. *J Am Acad Dermatol* 1988; **19:** 1089–95.

47 Berger CL, Morrison S, Chu A *et al.* Diagnosis of cutaneous T cell lymphoma by use of monoclonal antibodies reactive with tumour-associated antigens. *J Clin Invest* 1982; **70:** 1205–15.

48 Ralfkiaer E, Gatter KC, Wantzin GL. Immunohistological reactivity pattern of the anticutaneous T cell lymphoma antibody BE2. *Br J Dermatol* 1986; **114:** 677–84.

49 Picker LJ, Weiss LM, Medeiros LJ *et al.* Immunophenotypic criteria for the diagnosis of non-Hodgkin's lymphoma. *Am J Pathol* 1987; **128:** 181–99.

50 Medeiros LJ, Picker LJ, Abel EA *et al.* Cutaneous lymphoid hyperplasia. Immunologic characteristics and assessment of criteria recently proposed as diagnostic of malignant lymphoma. *J Am Acad Dermatol* 1989; **21:** 929–42.

51 Garcia CF, Weiss LM, Warnke RA, Wood GS. Cutaneous follicular lymphoma. *Am J Surg Path* 1986; **10:** 454–63.

52 Wirt DP, Grogan TM, Jolly CS *et al.* The immunoarchitecture of cutaneous pseudolymphoma. *Human Pathol* 1985; **16:** 492–510.

53 Clark DM, Boylston AW, Hall PA, Carrel S. Antibodies to T cell antigen receptor beta chain family detect monoclonal T cell proliferation. *Lancet* 1986; **2:** 835–37.

54 Jack AS, Boylston AW, Carrel S, Grigor I. Cutaneous T cell lymphoma cells employing a restricted range of T cell antigen receptor variable region genes. *Am J Pathol* 1990; **136:** 17–21.

55 Wood GS, Abel EA, Hoppe RT, Warnke RA. Leu 8 and Leu 9 antigen phenotype: immunologic criteria for the distinction of mycosis fungoides from inflammation. *J Am Acad Dermatol* 1986; **14:** 1006–13.

56 Ralfkiaer E, Lange Wantzin G, Mason D *et al* Phenotypic characterisation of lymphocyte subset in mycosis fungoides. Comparison with large plaque parapsoriasis and benign chronic dermatoses. *Am J Clin Path* 1985, **84:** 610–19.

57 Van der Putte SCJ, Toonstra J, van Wichen DF *et al.* Aberrant immunophenotypes in mycosis fungoides. *Arch Dermatol* 1988; **124:** 373–80.

58 Sperling M, Kaudewitz P, Braun-Falco O, Stein H. Reactivity of T cells in mycosis fungoides exhibiting marked epidermotrophism with monoclonal antibody HML-1 that defines a membrane molecule on human mucosal lymphocytes. *Am J Pathol* 1989; **134:** 955–60.

59 Palleson G, Hamilton-Dutoit SJ. Monoclonal antibody (HML-1) labelling of T cell lymphomas. *Lancet* 1989; **1:** 223.

60 McMillan E, Stoneking LE. Identification and possible significance of HNK-1 human lymphocytes, macrophages and non-neoplastic T cells in cutaneous lymphomas. *Am J Dermatopathol* 1987; **9:** 2–9.

61 Wood NL, Kitces EN, Blaylock WK. Depressed lymphokine activated killer cell activity in mycosis fungoides. A possible marker of aggressive disease. *Arch Dermatol* 1990; **126:** 907–13.

62 Nickoloff BJ, Griffiths CEM. Intraepidermoid but not dermal T lymphocytes are positive for a cell-cycle-associated antigen (Ki-67) in mycosis fungoides. *Am J Pathol* 1990, **136:** 261–5.

63 Ralfkiaer E, Stein H, Wantzin GL *et al.* Lymphomatoid papulosis. Characterisation of skin infiltrates by monoclonal antibodies. *Am J Clin Pathol* 1985; **84:** 587–93.

64 Kadin M, Nasu K, Sako D *et al.* Lymphomatoid papulosis. A cutaneous proliferation of activated helper T cells expressing Hodgkin's disease-associated antigen. *Am J Pathol* 1985; **119:** 315–25.

65 Kaudewitz P, Stein H, Plewig G *et al.* Hodgkin's disease followed by lymphomatoid papulosis. Immunophenotypic evidence for a close relationship between lymphomatoid papulosis and Hodgkin's disease. *J Am Acad Dermatol* 1990; **22:** 999–1006.

66 Macaulay WL. Lymphomatoid papulosis update. A historical update. *Arch Dermatol* 1989; **125:** 1387–9.

67 Kadin ME. Common activated helper T cell origin for lymphomatoid papulosis, mycosis fungoides and some types of Hodgkin's disease. *Lancet* 1985; **ii:** 864–5.

68 Slater DN, Rooney N, Bleehen S, Hamed A. The lymph node in mycosis fungoides: a light and electron microscopy and immunohistochemical study supporting the Langerhans' cell-retrovirus hypothesis. *Histopathology* 1985; **9:** 587–621.

69 Cerio R, Griffiths CEM, Cooper KD *et al.* Characterisation of factor XIIIa positive dermal dendritic cells in normal and inflamed skin. *Br J Dermatol* 1989; **121:** 421–31.

70 Vejlsgaard GL, Ralfkiaer E, Avnstorp C *et al.* Kinetics and characterisation of intercellular adhesion molecule-1 (ICAM-1) expression on keratinocytes in various inflammatory skin lesions and malignant cutaneous lymphomas. *J Am Acad Dermatol* 1989; **20:** 782–90.

71 Nickoloff BJ, Griffiths CEM, Baadsgaard O *et al.* Markedly diminished epidermal keratinocyte expression of intercellular adhesion molecule-1 (ICAM-1) in Sézary syndrome. *JAMA* 1989; **261:** 2217–21.

72 Weiss LM, Hu E, Wood GS *et al* Clonal rearrangements of T cell receptor genes in mycosis fungoides and dermatopathic lymphadenopathy. *New Engl J Med* 1985; **313:** 539–44.

73 Weiss LM, Wood GS, Hu E *et al.* Detection of clonal T cell receptor gene rearrangements in the peripheral blood of patients with mycosis fungoides/Sézary syndrome. *J Invest Dermatol* 1989; **92:** 601–4.

74 Bertness V, Kirsch I, Hollis G *et al.* T cell receptor gene rearrangements as clinical markers of human T cell lymphomas. *N Engl J Med* 1985; **313:** 534–8.

75 Waldmann TA, Davis MM, Bongiovanni KF, Korsmeyer SJ.

Rearrangements of genes for the antigen receptor on T cells as markers of lineage and clonality in human lymphoid neoplasms. *N Engl J Med* 1985; **313:** 776–82.

76 O'Connor NTJ. Cutaneous T cell lymphoma. In: The Genotypic analysis of human lymphoma. *MD Thesis*, Oxford University, 1986: 68–75.

77 Ralfkiaer E, O'Connor NTJ, Crick JA *et al.* Genotypic analysis of cutaneous T-cell lymphoma. *J Invest Dermatol* 1987; **88:** 762–5.

78 Bignon YJ, Roger H, Soutegrand P *et al.* Study of T cell antigen receptor gene rearrangement: a useful tool for the early diagnosis of mycosis fungoides. *Acta Derm Venereol (Stockh)* 1989; **69:** 217–22.

79 Peters MS, Thibodeau SN, White JW, Winkelmann RK. Mycosis fungoides in children and adolescents. *J Am Acad Dermatol* 1990; **22:** 1011–18.

80 Bunn PA, Whang-Peng J, Carney DN *et al.* DNA content analysis by flow cytometry and cytogenetic analysis in mycosis fungoides and Sézary syndrome: diagnostic and prognostic implications. *J Clin Invest* 1980; **65:** 1440–8.

81 Fuks ZY, Bagshaw MA, Farber EM. Prognostic signs and the management of mycosis fungoides. *Cancer* 1973; **32:** 1385–93.

82 Miller RA, Coleman CN, Fawcett HD *et al.* Sézary syndrome: a model for migration of T lymphocytes to skin. *N Engl J Med* 1980; **303:** 89–92.

83 Shackney SE, Schuette WH. Multicompartment analysis of cell proliferation and cell migration in the Sézary syndrome. *Hematological Oncology* 1983; **1:** 31–48.

84 Bunn PA, Edelson R, Ford SS, Shackney SE. Patterns of cell proliferation and cell migration in the Sézary syndrome. *Blood* 1981; **57:** 452–63.

85 Whittaker SJ, Smith NP, Jones RR, Luzzatto L. TCR gene analysis in cutaneous lymphomas and pseudolymphomas. In: *International Symposium in Cutaneous Lymphoma.* Copenhagen, Denmark, 1988; 44.

86 Laroche L, Mathieu-Mahul D Heterogeneity of T alpha chain gene rearrangement in cutaneous T cell lymphoma. In: *International Symposium in Cutaneous Lymphoma.* Copenhagen, Denmark, 1988, 11.

87 Bendelac A, O'Connor NTJ, Daniel M-T *et al.* Non-neoplastic circulating Sézary-like cells in cutaneous T cell lymphoma. Ultrastructural, immunologic and T cell receptor gene rearrangement studies. *Cancer* 1987; **60:** 980–6.

88 Ho VC, Baadsgaard O, Elder JT *et al.* Genotypic analysis of T cell clones derived from cutaneous T cell lymphoma lesions demonstrating selective growth of tumour infiltrating lymphocytes. *J Invest Dermatol* 1990; **95:** 4–8.

89 Weiss LM, Wood GS, Trela M *et al.* Clonal T cell populations in lymphomatoid papulosis. Evidence of a lymphoproliferative origin for a clinically benign disease. *N Engl J Med* 1986; **315:** 475–9

90 Kadin ME, Vonderheid EC, Sako D *et al.* Clonal composition of T cells in lymphomatoid papulosis. *Am J Pathol* 1987; **126:** 13–18.

91 Kaudewitz P, Herbst H, Anagnostopoulos I *et al.* Lymphomatoid papulosis followed by large cell lymphoma: immunophenotypical and genotypical analysis. *Br J Dermatol* 1991; **124** (in press).

92 Whittaker SJ, Smith NP, Russell Jones R, Luzzatto L. Lymphomatoid papulosis—a genotypic study. *Br J Dermatol* 1989; **121** (Suppl. 34): 21–2.

93 Cleary ML, Nalesnik MA, Shearer WT, Sklar J. Clonal analysis of transplant-associated lymphoproliferations based on the structure of the termini of the Epstein–Barr virus. *Blood* 1988; **72:** 349–52.

94 Arnold A, Crossman J, Bakhshi A *et al.* Immunoglobulin-gene rearrangements as unique clonal markers in human lymphoid neoplasms. *New Engl J Med* 1983; **309:** 1593–9.

95 Berti E, Gianotti R, Alessi E, Caputo R. Primary cutaneous germinal centre lymphomas. In: *International Symposium in Cutaneous Lymphoma.* Copenhagen, Denmark, 1988; 28.

96 Weiss LM, Wood GS, Ellisen LW *et al.* Clonal T cell populations in pityriasis lichenoides et varioliformis acuta (Mucha-Habermann disease). *Am J Pathol* 1987; **126:** 417–21.

97 Wechsler J, Bagot M, Henni T *et al.* Cutaneous pseudolymphomas. In: *International Symposium in Cutaneous Lymphoma.* Copenhagen, Denmark, 1988; 24.

98 Wood GS, Ngan B-Y, Tung R *et al.* Clonal rearrangements of immunoglobulin genes and progression to B cell lymphoma in cutaneous lymphoid hyperplasia. *Am J Pathol* 1989; **135:** 13–19.

99 Flynn KJ, Dehner LP, Kazimiera J *et al.* Regressing atypical histiocytosis. A cutaneous proliferation of atypical neoplastic histiocytes with unexpectedly indolent biologic behaviour. *Cancer* 1982; **49:** 959–70.

100 Isaacson PG, Spencer J, Connolly CE *et al.* Malignant histiocytosis of the intestine. *Lancet* 1985; **ii:** 688–91.

101 Liebow AA, Carrington CB, Friedman PJ. Lymphomatoid granulomatosis. *Human Pathology* 1972; **3:** 457–558.

102 Troussard X, Galateau F, Gaulard P *et al.* Lymphomatoid granulomatosis in a patient with acute myeloblastic leukaemia in remission. *Cancer* 1990; **65:** 107–111.

103 Wood GS, Weiss LM, Hu C-H *et al* T cell antigen deficiencies and clonal rearrangements of T cell receptor genes in pagetoid reticulosis (Woringer–Kolopp disease) *N Engl J Med* 1988; **318:** 164–7.

104 LeBoit PE, Beckstead JH, Bond B *et al.* Granulomatous slack skin: clonal rearrangement of the T cell receptor beta gene is evidence for the lymphoproliferative nature of a cutaneous elastolytic disorder. *J Invest Dermatol* 1987; **89:** 183–6.

105 Petroff N, Koger O'N W, Fleming MG *et al.* Malignant angioendotheliomatosis; an angiotropic lymphoma. *J Am Acad Dermatol* 1989; **21:** 727–33.

106 Sepp N, Fritsch P, Schuler G *et al.* Detection of clonal beta T cell receptor gene rearrangement in proliferative angioendotheliomatosis. In: *International Symposium in Cutaneous Lymphoma.* Copenhagen, Denmark, 1988; 10.

107 Berger CL, Eisenberg A, Soper L *et al.* Dual genotype in cutaneous T-cell lymphoma: immunoglobulin gene rearrangement in clonal T cell malignancy. *J Invest Dermatol* 1988; **90:** 73–7.

108 Weiss LM, Picker LJ, Grogan TM *et al.* Absence of clonal beta and gamma T cell receptor gene rearrangements in a subset of peripheral T cell lymphomas. *Am J Pathol* 1988; **130:** 436–42.

109 Aisenberg AC, Krontiris TG, Mak TW *et al.* Rearrangement of the gene for the beta chain of the T cell receptor in T cell chronic lymphocytic leukaemia and related disorders. *N Engl J Med* 1985; **313:** 529–33.

110 Cossman J, Upperkamp M, Sundeen J *et al.* Molecular genetics and the diagnosis of lymphoma. *Arch Path Lab Med* 1988; **112:** 117–27.

111 Messenger AG, Marshall TL, Summerly R. A case of lymphomatoid papulosis and systemic lymphoma. *Br J Dermatol* 1981; **104:** 77–82.

112 Mielka V, Wolff H, Winzer M, Sterry W. Localised and disseminated pagetoid reticulosis. Diagnostic immunophenotypical findings *Arch Dermatol* 1989; **125:** 402–6.

113 Slater DN. Clonal dermatoses a conceptual and diagnostic dilemma. *J Pathol* 1990; **162:** 1–4.

114 Stamenkovic I, Stegagno M, Wright KA *et al.* Clonal dominance

among T lymphocyte infiltrates in arthritis. *Proc Natl Acad Soc* 1988; 85: 1179–83.

115 Bignon YJ, Souteyrand P. Genotyping of cutaneous T cell lymphomas and pseudolymphomas. In. *Current Problems in Dermatology. Vol 19. Cutaneous Lymphoma.* (Van Vloten WA, Willhemze R, Vejlsgaard GL, Thompson K, eds). Switzerland. Karger, 1990; 114–23.

116 Griesser H, Feller AC, Sterry W. T cell receptor and immunoglobulin gene rearrangement in cutaneous T cell rich pseudolymphomas. *J Invest Dermatol* 1990; 95: 292–5.

117 Eckert F, Kaudewitz P, Hefner H, Burg G. Disseminierte follikulare pseudolymphome der Haut-Klinik and Histopathologic. *Zbl Haut Gshlkr* 1988; 154: 614.

118 Weiss LM, Warnke RA, Sklar J, Cleary ML. Molecular analysis of the t (14:18) chromosome translocation in malignant lymphoma. *N Engl J Med* 1987; 317: 1185–9.

119 Whang-Peng J, Bunn P, Knutsen T *et al.* Cytogenetic abnormalities in patients with cutaneous T cell lymphoma. *Cancer Trect Report* 1979; 63: 575–80.

120 Johnson GA, Dewald GW, Strand WR, Winkelmann RK. Chromosome studies in 17 patients with the Sézary syndrome. *Cancer* 1985; 55: 2426–33.

121 Maeda K, Yamana K, Takahashi H, Jimbow K. Dual surface markers and HTLV-1 proviral DNA in a cutaneous tumour nodule in a case of adult T cell leukaemia/lymphoma. *Br J Dermatol* 1987; 117: 561–8.

122 Takahashi K, Tanaka T, Fujita M *et al.* Cutaneous type adult T cell leukaemia/lymphoma. *Arch Dermatol* 1988; 124: 399–404.

123 Tanaka T, Takahashi K, Ideyama S *et al.* Demonstration of clonal proliferation of T lymphocytes in early neoplastic disease. *J Am Acad Dermatol* 1989; 21: 218–23.

124 Wantzin GL, Saxinger WC, Woods A *et al.* Human T cell leukaemia virus in cutaneous T cell lymphoma in Denmark. *Acta Derm Venereol (Stockh)* 1984; 64: 395–9.

125 Ranki A, Crohn K. Cross-reacting antibodies to GAG protein of HTLV-1 and HTLV-3 in patients with mycosis fungoides or its prodrome large plaque parasoriasis. In: *Viruses in Human Cancer* (Gallow RC, Haseltine W, Klein G, Zurhausen H, ed). New York: Alan R Liss, 1987; 43–57.

126 Detmar M, Pauli G, Anagnostopoulos I *et al.* A case of mycosis fungoides associated with human T cell lymphotrophic virus type 1. *Br J Dermatol* 1991; 124: 198–202.

127 Lee LA, Huff JC, Edmond BJ *et al.* Identification of herpes virus simplex antigen and DNA in lesions of mycosis fungoides. *J Invest Dermatol* 1983; 80: 333.

128 Josephs SF, Salahuddin SZ, Ablashi DV *et al.* Genomic analysis of the human B lymphotrophic virus (HBLV). *Science* 1986; 234: 601–3.

129 Jones JF, Shurin S, Abramowsky C *et al.* T cell lymphomas containing Epstein–Barr viral DNA in patients with chronic Epstein–Barr virus infections. *N Engl J Med* 1988; 318: 733–40.

130 Weiss LM, Strickler JG, Warnke RA *et al.* Epstein–Barr viral DNA in tissues of Hodgkin's disease. *Am J Pathol* 1987; 129: 86–91.

131 Herbst H, Niedobitek G, Kneba M *et al.* High incidence of Epstein–Barr virus genomes in Hodgkin's disease. *Am J Pathol* 1990; 137: 13–18.

132 Lee PYP, Charley M, Tharp M *et al.* Possible role of Epstein–Barr virus infection in cutaneous T cell lymphoma. *J Invest Dermatol* 1990; 95: 309–12.

133 Harabuchi Y, Yamaraka N, Kataura A *et al.* Epstein–Barr virus in nasal T cell lymphomas in patients with lethal midline granuloma. *Lancet* 1990; 1: 128–30.

134 Wright PA, Wynford-Thomas D. The polymerase chain reaction: Miracle or mirage? A critical review of its uses and limitations in diagnosis and research. *J Pathol* 1990; 162: 99–117.

135 McCarthy KP, Sloane JP, Wiedemann LM. Rapid method for distinguishing clonal from polyclonal B cell populations in surgical biopsy specimens. *J Clin Pathol* 1990; 43: 429–32.

136 Goudie RB. Hypothesis. A strategy for demonstrating the clonal origin of small numbers of T lymphocytes in histopathological specimens. *J Pathol* 1989; 158: 261–5.

137 Bunker CB, Whittaker S, Luzzatto *et al.* Indolent cutaneous prodrome of fatal HTLV-1 infection. *Lancet* 1990; i: 426.

138 Lambert WC. Beyond hybridomas. Cell identification by *in situ* nucleic acid hybridisation. *Am J Dermatopathol* 1988; 10: 144–54.

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Substance P antagonist inhibits immediate and delayed type cutaneous hypersensitivity reactions

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Summary The role of substance P (SP) in allergic reactions of the skin was investigated. Spantide, a competitive inhibitor of SP, was injected intracutaneously into the volar aspect of the forearm prior to the following challenges: benzalkonium chloride (irritant delayed reaction), tuberculin (immunological delayed reaction), UVB irradiation, benzoic acid (non-immunological contact urticaria), different food allergens and latex (in patients with immunological contact urticaria). Only the immunological reactions of contact urticaria and the reaction to tuberculin were suppressed by the SP antagonist, indicating that SP is involved in their pathogenesis.

Substance P (SP) is an important inflammatory mediator in the skin[1] that is localized in peripheral sensory nerve endings. It coexists with another tachykinin, neurokinin A (NKA),[2] and with calcitonin gene-related peptide (CGRP).[3]

Increased local concentrations of SP were found in tissue fluid from spontaneous and suction-induced blisters in inflamed skin. High levels were also found in eczema and pemphigoid,[4] two immunologically different diseases. When injected intradermally SP induces a 'triple response' with local erythema, a rapidly spreading flare and a slowly developing weal.[5] The flare response to SP is greatly inhibited by antihistamines,[5] local anaesthetics[6,7] and partly by drugs that inhibit the synthesis of prostaglandins.[8] SP is known to release histamine from mast cells.[6]

There is increasing evidence that the nervous system can modulate immune responses. Nerve endings occur in the vicinity of various structures of the immune system.[9] Lymphoid cells have receptors for SP[10] and SP enhances the proliferation of T lymphocytes[10–12] and increases the synthesis of the immunoglobulins IgA and IgM.[12–14]

In a previous study we described the suppression of allergic contact dermatitis after pretreatment with a competitive inhibitor (Spantide) of SP.[15] The aim of the present study was to investigate whether SP can modulate delayed and immediate inflammatory reactions in man. The SP antagonist, Spantide, was used to examine the role of SP in these reactions.

Methods

The study involved 23 subjects, 16 healthy volunteers (10 females, age range 17–50 years, mean age 34 ± 11 years) and seven patients with contact urticaria (six females, mean age 27 ± 9, three atopics) (Table 1). Informed consent was obtained in all cases.

In order to explore the properties of Spantide,[16] SP (50 pmol) and histamine (5.0 nmol) were injected intracutaneously (i.c.) 75–90 min after a preinjection of Spantide in six subjects. Spantide (800 pmol, 0·05 ml) was injected i.c. on the volar side of one forearm and physiological saline (0·05 ml) on the other forearm. The choice of right or left arm for the injection of Spantide was random. The flare reaction to i.c. SP and histamine was recorded after 5 min and the weal after 15 min, when maximal.

The following experiments were performed on the 16 healthy volunteers 10 min after the injection of Spantide if not otherwise stated. In eight subjects physiological saline solution was used on the control forearm and in the other eight there was no pretreatment on the control side.

Irritant delayed reaction

Benzalkonium chloride 1% in water solution was applied to the skin of the pretreated sites by using Finn chambers® on Scanpor®. The compound was applied for 48 h and the reaction was examined after a further 24 h.

Table 1. Details of patients with contact urticaria

Case no.	Age (years)	Sex	Agents producing contact urticaria	Comments
1	21	M	Tomato, beef	
2	31	F	Tomato, kiwi fruit, shrimp	Allergic positive patch test to nickel sulphate, balsam of Peru, fragrance mix
3	19	F	Shrimp, fish, flour, egg white and yolk	History of atopic dermatitis
4	22	F	Flour	History of atopic dermatitis
5	46	F	Latex	
6	29	F	Shrimp	History of atopic dermatitis
7	23	F	Tomato	

Immunological delayed reaction: tuberculin reaction

The pretreated sites were injected i.c. with 0·1 ml of 2 TU PPD (Statens Serum Institut, Copenhagen, Denmark). The response area was estimated after 72 h.

Ultraviolet evoked reaction

Erythema was induced on the pretreated skin area using ultraviolet light from a 150 W xenon arc lamp (Osram XBO) giving a high output of UVB. The exposure time was 16–25 s corresponding to about 2–4 MED (minimal erythema dose). The inflammatory response was evaluated after 24 h.

Non-immunological contact urticaria (NICU)

Benzoic acid, 5%, dissolved in petrolatum was applied to the skin by Finn chambers® or Scanpor® for 45 min. The urticarial reaction was registered immediately after removal of the chambers. In nine subjects benzoic acid was applied 10 min after pretreatment with Spantide or physiological saline and in the other seven, applied 75 min after the pretreatment.

Immunological contact urticaria

The following tests were performed on the non-diseased skin on the volar aspect of the forearm of the patients with contact urticaria. Seven patients were tested with the following agents (Table 1): Patient 1, tomato and beef; Patient 2, tomato, kiwi fruit and shrimp; Patient 3, shrimp, fish, flour, egg white and yolk; Patient 4, flour; Patient 5, latex; Patient 6, shrimp; Patient 7, tomato. Seventy-five minutes after injection with Spantide or physiological saline the 1 cm^2 skin area was scratched with a blood lancet and the pieces of fresh food, fruits and latex glove were applied by van der Bend square chambers® for 20 min. Evaluation was made 5–15 min after removal of the chambers.

Evaluation

The flare and weal responses to SP and histamine were evaluated planimetrically.[7] A score system was used for the reactions to UVB and benzalkonium chloride which were evaluated as follows: 1, erythema; 2, erythema and infiltration; 3, erythema and strong infiltration; 4, erythema, strong infiltration and vesicles. The infiltrated responses to tuberculin, benzoic acid and immunological contact urticaria were scored as follows with regard to the mean of two angle diameters: 1, <1 cm; 2, $<1{\cdot}5$ cm; 3, $<2{\cdot}5$ cm; 4, $>2{\cdot}5$ cm.

The Wilcoxon signed-rank test and Wilcoxon rank-sum test were used for statistical analysis.

Results

Properties of Spantide

Spantide induced a triple response and itch. The itch subsided after a few minutes and the flare and weal reactions disappeared after 75–90 min. The results are presented as a comparison of the median of the area from six subjects. Spantide inhibited the flare of substance P to 23% ($P=0{\cdot}025$) and weal to 42% of the controls ($P=0{\cdot}025$). The flare reaction to histamine after the preinjection of Spantide was reduced to 73% ($P=0{\cdot}025$) (Fig. 1) and the weal was unaffected ($P>0{\cdot}05$) (Fig. 2).

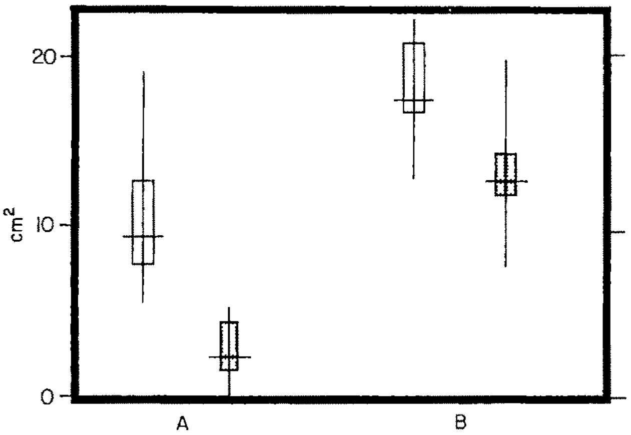

Figure 1. The flare reaction to i.c. injection of (A) SP (50 pmol) and (B) histamine (50 nmol) after i.c. pretreatment with saline (□) and Spantide (800 pmol) (▦). The boxes express half of all responses, the vertical lines represent the range of the data and the horizontal lines show the median values.

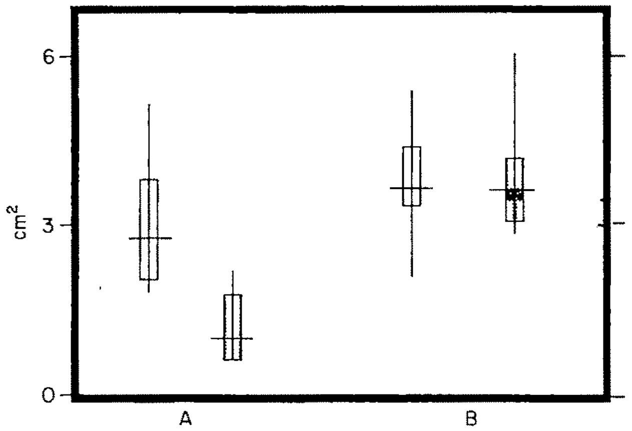

Figure 2. The weal reaction to i.c. injection of (A) SP (50 pmol) and (B) histamine (50 nmol) after i.c. pretreatment with saline (□) and Spantide (800 pmol) (▦). The boxes express half of all responses, the vertical lines represent the range of the data and the horizontal lines show the median values.

The results from the experiments on the influence of Spantide on inflammation arising from challenge with different agents are presented in Table 2 and Figure 3.

Non-immunological contact urticaria (NICU, benzoic acid)

The first nine subjects to be tested were challenged with benzoic acid 10 min after the injection of Spantide. The flare and weal caused by Spantide was still present and this made evaluation of NICU impossible. These results were consequently excluded from the study. The remaining seven patients were challenged with benzoic acid 75–90 min after the injection of Spantide or physiological saline. One subject did not respond and the others showed the same reactions on both sites.

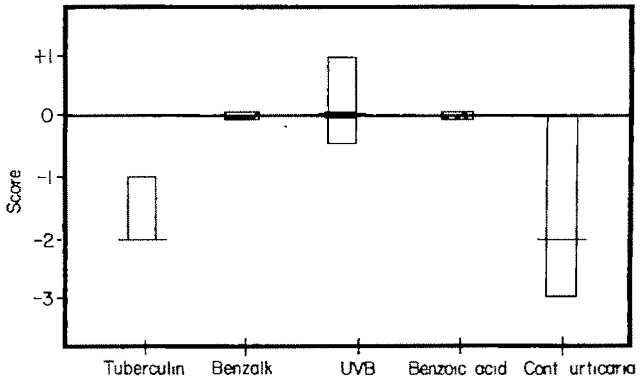

Figure 3. Response to challenges: The vertical axis shows the median of the differences in the score between the forearm pretreated with Spantide and the control forearm. The boxes reveal the confidence interval (95%).

Table 2. The differences in the responses (score) to different challenges between the site pretreated with Spantide and the site pretreated with physiological saline solution

No.	Tuberculin	Benzalkonium chloride	UVB	Benzoic acid	Contact urticaria
1	−1	3	0	–	0
2	−2	−1	Neg	–	0
3	−1	0	0	–	0
4	Neg	−2	0	–	−1
5	−1	0	0	–	−2
6	−2	−1	0	–	−2
7	−2	2	Neg	–	−3
8	Neg	NT	0	–	−3
9	−1	4	0	–	−3
10	−3	0	−0·5	0	0
11	−3	0	−1·5	0	−2·5
12	−3	0	−0 5	0	Neg
13	−3	0	−1	0	Neg
14	Neg	0	0	0	Neg
15	−3	0	0	0	
16	−2	0	0	Neg	

NT, not tested; Neg, no response; –, excluded.

Immunological contact urticaria

Two subjects were excluded as they did not respond to the allergens (tomato and beef). The patients who responded to Spantide had a history of atopic disease.

UVB-evoked erythema

This was unaffected by pretreatment with Spantide.

Delayed reactions

Spantide suppressed the immunological reactions (Fig. 3). The immunological reaction (tuberculin) was significantly inhibited ($P=0{\cdot}001$) when compared with the non-immunological reaction (benzalkonium chloride) using the Wilcoxon signed-rank test (Fig. 3).

Immediate reactions

The immunological reaction (contact urticaria) was significantly inhibited ($P=0{\cdot}035$) compared with the non-immunological reaction (NICU) using the Wilcoxon rank-sum test (Fig. 3).

There were no differences between the control group without pretreatment and the control group with pretreatment using physiological saline.

Discussion

The aim of this study was to investigate whether substance P (SP) can modulate immediate and delayed reactions in human skin of immunological and non-immunological origin. The study shows that only the immunological reactions (immunological contact urticaria and tuberculin reaction) were suppressed by the SP antagonist Spantide, indicating either that SP participates in these reactions or that Spantide possesses inhibitory immunological properties.

When injected intradermally, SP induces mast-cell degranulation and stimulates sensory nerves to release SP, NKA and CGRP.[7] NKA and histamine induce vasodilatation and increased permeability which gives flare and weal. The flare of SP and NKA but not that of CGRP is inhibited by antihistamines.[7] The weal response of SP is not inhibited by pretreatment with antihistamines, the histamine liberator compound 48/80, local anaesthetics, inhibitors of prostaglandin synthesis, 5-HT-antagonist or atropine (unpublished data).

Our study showed that Spantide partially inhibited (73%) the flare caused by histamine and left the weal unchanged while it suppressed the flare caused by SP to 23% and the weal to 42% of the controls. This indicates that Spantide directly inhibits the response to SP and to a lesser degree by inhibiting histamine release.

Non-immunological contact urticaria (NICU) is due to the release of vasoactive substances that do not involve immunological processes.[17] Oral antihistamine therapy and pretreatment with compound 48/80 do not influence NICU (unpublished data). Local anaesthesia with lignocaine decreases NICU,[17] indicating involvement of sensory nerves. In allergic contact urticaria specific IgE has been found,[18,19] indicating that an antibody–antigen response is involved. IgG and IgM may also contribute by activation through the classical pathway of the complement cascade.[18] The synthesis of IgA and IgM has been found to be increased by SP.[12–14] In humans allergic to rats the flare and itch but not the weal have been suppressed by pretreatment with repeated applications of capsaicin[20] which releases neuropeptides from sensory nerves. Both types of contact urticaria seem to involve nerve fibres. However, in our study only the immunological contact urticaria was suppressed by pretreatment with Spantide, suggesting that SP is involved in the pathogenesis of the immediate allergic reaction.

Irritant delayed reactions were unaffected by pretreatment with Spantide and only the tuberculin reaction was suppressed. This is consistent with the results of a previous study of nickel allergy[15] which also represents a T lymphocyte-mediated type IV reaction. Pretreatment with Spantide suppressed the symptoms of contact urticaria in atopic patients. SP may play a role in both immediate and delayed reactions.

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References

1 Pernow B. Substance P. *Pharmacol Rev* 1983; **35**: 85–141.

2 Sundler F, Brodin E, Ekblad E *et al.* Sensory nerve fibers: distribution of substance P, neurokinin A and calcitonin gene related peptide. In: *Tachykinin Antagonists*. Elsevier Science Publishers BV. 1984; 3–14.

3 Wallengren J, Ekman R, Sundler F. Occurrence and distribution of neuropeptides in the human skin. *Acta Derm Venereol (Stockh)* 1987; **67**: 185–92.

4 Wallengren J, Ekman R, Möller H. Substance P and vasoactive intestinal peptide in bullous and inflammatory skin disease. *Acta Derm Venereol (Stockh)* 1986; **66**: 23–8.

5 Hägermark Ö, Hökfelt T, Pernow B. Flare and itch induced by substance P in human skin. *J Invest Dermatol* 1978; **71**: 233–5.

6 Foreman JC, Jordan CC, Oehme, P, Renner H. Structure activity relationships for some substance P related peptides that cause wheal and flare reactions in human skin. *J Physiol* 1983; **335**: 449–65.

7 Wallengren J, Håkanson R. Effects of substance P, neurokinin A and calcitonin gene-related peptide in human skin and their

involvement in sensory nerve-mediated responses. *Eur J Pharmacol* 1987; **143**: 267–73.

8 Ståhle M, Hagermark Ö. Separate effects of topical indomethacin on the itch response and on the flare reaction induced by histamine in human skin. *Acta Derm Venereol (Stockh)* 1985; **65**: 340–2.

9 Blalock JE. The immune system as a sensory organ. *J Immunol* 1984; **132**: 1067–70.

10 Payan DG, Brewster DR, Missirian-Bastian A, Goetzl EJ. Substance P recognition by a subset of human T lymphocytes. *J Clin Invest* 1984; **74**: 1532–9.

11 Payan DG, Brewster DR, Goetzl EJ. Specific stimulation of human T lymphocytes by substance P. *J Immunol* 1983; **131**: 1613–15.

12 Bienenstock J, Denburg J, Scicchitano R *et al.* Role of neuropeptides, nerves and mast cells in intestinal immunity and physiology. *Monogr Allergy* 1988; **24**: 134–43.

13 Stanisz AM, Befus D, Bienenstock J. Differential effects of vasoactive intestinal peptide, substance P and somatostatin on immunoglobulin synthesis and proliferations by lymphocytes from Peyer's patches, mesenteric lymph nodes, and spleen. *J Immunol* 1986; **136**: 152–6.

14 Scicchitano R, Bienenstock J, Stanisz AM. *In vivo* immunomodulation by the neuropeptide substance P. *Immunology* 1988; **63**: 733–5.

15 Wallengren J, Möller H Some neuropeptides as modulators of experimental contact allergy. *Contact Dermatitis* 1988; **19**: 351–4.

16 Folkers K, Håkanson R, Horig J *et al.* Biological evaluation of substance P antagonists *Br J Pharmacol* 1984; **83**: 449–56.

17 Lahti A. Non-immunologic contact urticaria. *Acta Derm Venereol (Stockh)* 1980; **60** (Suppl 91): 1–49.

18 von Krogh G, Maibach HI. The contact urticaria syndrome—an updated review. *J Am Acad Dermatol* 1981; **5**: 328–42.

19 Wrangsjö K, Wahlberg JE, Axelsson IGK. Ig-E mediated allergy to natural rubber in 30 patients with contact urticaria. *Contact Dermatitis* 1988; **19**: 264–71.

20 Lundblad L, Lundberg JM, Änggård A, Zetterström O. Capsaicin pretreatment inhibits the flare component of the cutaneous allergic reaction in man. *Eur J Pharmacol* 1985; **113**: 461–2.

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Hailey–Hailey disease: a widespread abnormality of cell adhesion

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Summary Suction has been used to investigate cell adhesion in clinically normal skin in Hailey–Hailey disease. We have demonstrated that there is a widespread subclinical abnormality in keratinocyte adhesion in this disease. There may be a synthesis of functionally deficient adhesion junctions, increased breakdown of adhesion junctions or abnormalities in other adhesion proteins in the epidermis in Hailey–Hailey disease. The findings contrast with those in Darier's disease in which abnormal cell adhesion was only demonstrable in clinically involved skin.

Hailey–Hailey disease (benign familial chronic pemphigus) is a dominantly inherited disorder characterized by the development of flexural erosions, blisters and warty papules.[1] The disease has a fluctuating course, but in most patients remains localized to the neck, axillae and groins. Typical histological features include suprabasal epidermal cell separation (acantholysis) with the formation of vesicles or bullae and a characteristic dyskeratosis.[2] Ultrastructural studies have demonstrated breakdown of the complexes between desmosomes and the keratinocyte tonofilaments.[2–4]

Lesions are induced or exacerbated by friction and sweating and although the uninvolved skin is clinically and microscopically normal, there must be a subclinical abnormality that predisposes to the development of erosions and vesiculation. Sellotape stripping, friction, freezing and irritants induce acantholysis in clinically normal skin in patients with Hailey–Hailey disease,[5] but because these also cause both inflammation and damage to keratinocytes, the pathogenesis of the acantholysis is uncertain. Suction places the cell junctions under tension without damaging the keratinocytes or causing inflammation[6,7] and in normal individuals suction blisters are formed by cleavage through the dermo-epidermal junction. The suction technique is painless, reproducible and easy to perform. Suction blisters in pemphigus vulgaris, an immunologically mediated acantholytic disease have established there was abnormal epidermal cell adhesion in the clinically normal skin,[8] whereas in the inherited dermatosis, Darier's disease, abnormal cell adhesion was only demonstrable in the involved skin.[9]

Hailey–Hailey disease has many clinical and pathological similarities to Darier's disease and occasionally the diseases are difficult to differentiate.[10] The coexistence of the conditions has been reported.[11] The aims of this study were to investigate the effect of suction on the epidermis in Hailey–Hailey disease and to establish whether suction could be used to differentiate Hailey–Hailey disease from Darier's disease.

Methods

Patients

Approval was obtained from the Central Oxford Research Ethical Committee and informed consent was obtained from the patients. Three unrelated adult males with Hailey–Hailey disease were investigated. The patients, aged 40, 53 and 54 years, had family histories of Hailey–Hailey disease and clinically typical disease involving the neck, axilla and groins. The diagnosis had been confirmed histologically. Two patients used topical corticosteroids only on affected skin and the third patient, with very mild disease, was not on any treatment.

Suction blisters

Perspex suction cups, with diaphragms perforated by five 6-mm holes, were placed on the flexor surface of clinically normal forearm skin. This skin had never been affected by the disease. Blisters were raised as described

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in previous studies.[6–9] The pressure within the cups was maintained at 200 mmHg below atmospheric pressure using an Eschmann VP 50 suction unit (Eschmann Bros and Walsh Ltd, Lancing, W. Sussex, U.K.). As soon as a blister formed, the experiment was terminated. Suction was discontinued and punch biopsies were taken under local anaesthesia. Biopsies were fixed in either 10% formalin or 4% glutaraldehyde and processed for light or electron microscopy, respectively.

Results

The time to blister formation was approximately 120 min.

Light microscopy

The epidermis adjacent to the area of blister formation was histologically normal. Areas of subepidermal blister formation were present in each biopsy, but there was also marked intraepidermal keratinocyte separation and the blisters contained acantholytic cells (Fig. 1). There was no inflammation in the epidermis or the dermis.

Electron microscopy

There was widening of the intercellular spaces between suprabasal cells and this was associated with stretching and fragmentation of desmosomes (Fig. 2). Cell membranes were intact and there was no reduction in desmosome numbers. Keratin intermediate filaments were evident, often encircling the nucleus. There was some vacuolization of the nucleus and cytoplasm.

Discussion

We have used a suction blister technique to investigate keratinocyte adhesion in the clinically normal forearm skin of patients with Hailey–Hailey disease. Suction caused breakdown of adhesions between the keratinocytes with vesiculation and acantholysis.

Burns, irritant dermatitis, allergic contact dermatitis and local infection also cause acantholysis in Hailey–Hailey disease,[5] but in these conditions there is damage to the keratinocytes and inflammation. Suction places the cell junctions under tension without damaging the keratinocytes or causing inflammation. Normal skin,

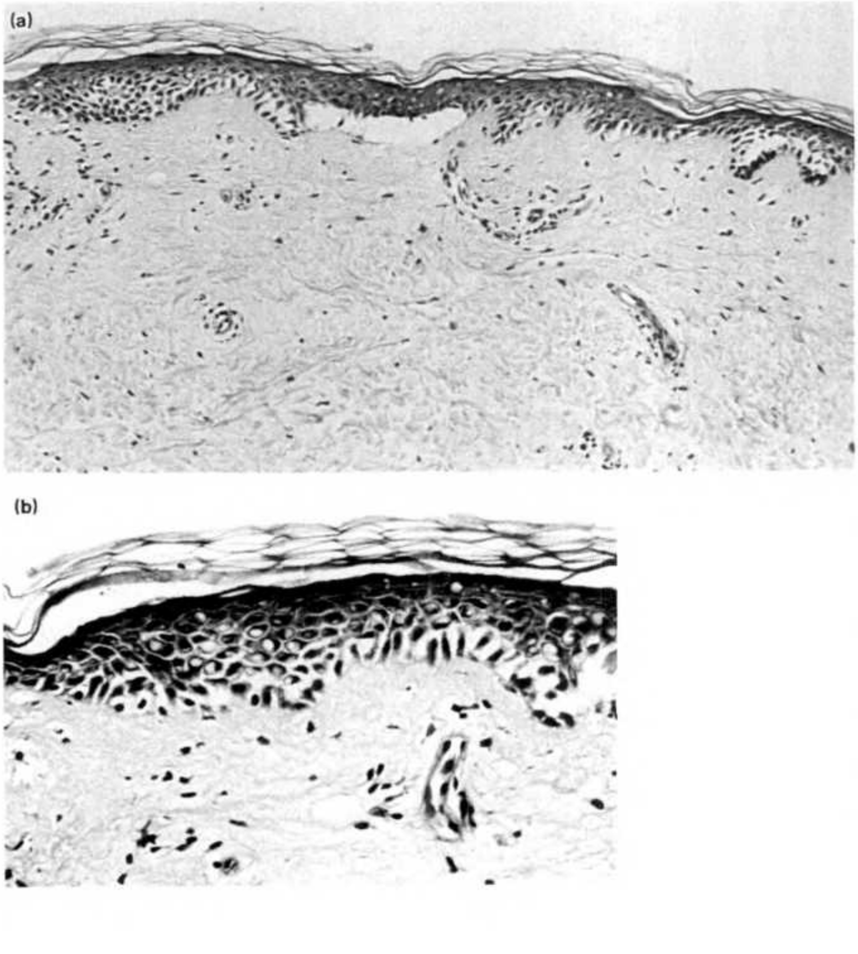

Figure 1. (a) Subepidermal and intraepidermal cleavage in a biopsy from a suction blister on the uninvolved forearm skin of a patient with Hailey–Hailey disease. Intraepidermal separation gives the epidermis the 'dilapidated brick wall' appearance which is characteristic of Hailey–Hailey disease. There is no inflammatory response in the dermis. (Haematoxylin and eosin, × 116). (b) Higher magnification of part of the epidermis showing loss of adhesion between suprabasal keratinocytes in addition to basal keratinocyte separation from the basement membrane zone. There is also vacuolization within the cytoplasm of the keratinocytes. (Haematoxylin and eosin, × 476).

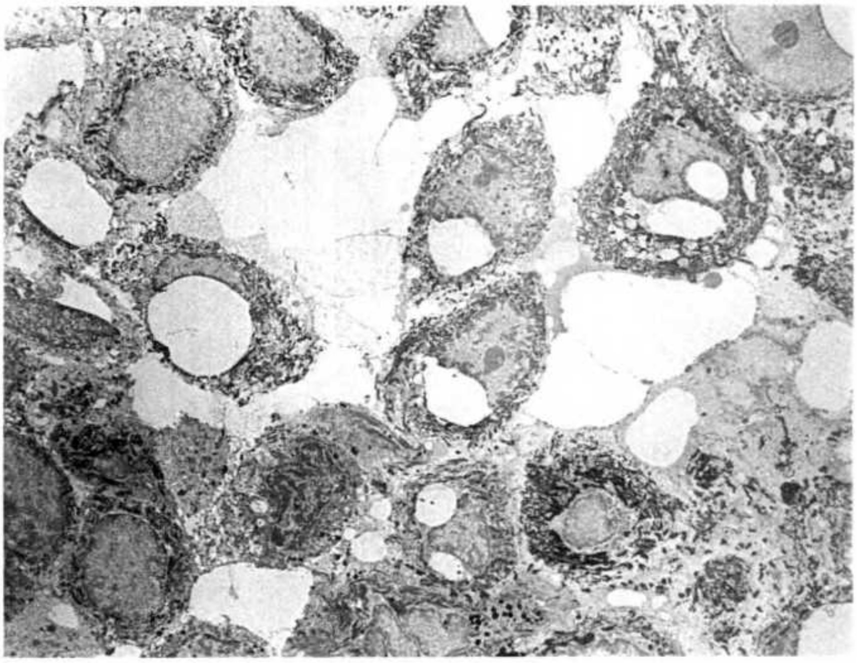

Figure 2. Low-power electron micrograph showing rupture of the demosomes between adjacent cells and perinuclear vacuolization of the cytoplasm of a keratinocyte in a suction blister on the uninvolved forearm skin of a patient with Hailey–Hailey disease (×2310).

when subjected to suction, splits through the lamina lucida to form a subepidermal blister. The blister roof is formed by the intact epidermis and there is no inflammation or autolysis.[6,7] Ultrastructural studies of suction blisters have confirmed that there is minimal damage to the keratinocytes in the detached epidermis. The desmosomes, cell membranes and the keratin intermediate filaments appear intact and there is little widening of the intercellular spaces.[6,7]

The sequence of events leading to acantholysis in Hailey–Hailey disease is uncertain. Ultrastructural studies have demonstrated dissolution of desmosome–keratin filament complexes, clumping of keratin filaments and alterations in the plasma membrane of keratinocytes.[2–4] Our results have demonstrated that there is a widespread intrinsic weakness in suprabasal keratinocyte adhesion which may be revealed by suction.

In Darier's disease, unlike Hailey–Hailey disease, suction did not show intraepidermal weakness either in distant clinically normal skin or in uninvolved skin adjacent to foci of disease.[9] Therefore the suction technique may be a useful investigative tool when it is not possible to differentiate the diseases solely on the basis of clinical or histological criteria.

Keratinocyte adhesion is dependent on the presence of specialized junctions, adherens-type junctions[12,13] and desmosomes,[13] at interfaces between cells as well as the presence of adhesion molecules such as the calcium-dependent family of glycoproteins, the cadherins.[14] The junctions interact with and anchor specific cytoskeletal filaments. Actin microfilaments insert into the adherens-type junctions,[13] which are enriched with vinculin, while keratin intermediate filaments insert into the cytoplasmic plaques of desmosomes,[13] which contain specific desmosomal proteins and glycoproteins. Friction, sweating and infection precipitate acantholytic lesions in patients with Hailey–Hailey disease[1] but it is not known what predisposes to this breakdown in epidermal cell adhesion. Desmosomes appear normal in intact epidermis and immunocytological studies have not revealed any deficiency in the major desmosomal proteins or glycoproteins,[15,16] but there may be synthesis of functionally deficient desmosomes. Alternatively there may be abnormalities in adherens-type junctions,[16] adhesion molecules such as cadherin-E or the structure and function of the cytoskeleton.

The control of cell attachment is incompletely understood, but activators of proteolysis co-localize on the cell surface with proteins such as vinculin which mediate adhesion.[17] There is variation in the sensitivity of desmosomes to splitting agents. Desmosomes formed by basal cells are preferentially split by plasmin[18,19] and plasminogen activation has been implicated in the pathogenesis of acantholysis in pemphigus.[19,20] In normal skin plasminogen is expressed in association with basal cells[21,22] but in skin affected by Hailey–Hailey disease, plasminogen is also detected in acantholytic suprabasal cells (S.M.Burge, unpublished observations). The acantholytic cells derived from explant cultures of skin from patients with Hailey–Hailey disease express both plasminogen and the plasminogen activator urokinase (S.M.Burge, unpublished observations). Activation of plasminogen with breakdown of cell attachments may contribute to acantholysis in Hailey–Hailey disease. Lesions develop predominantly at sites of friction and we have demonstrated that the keratinocyte adhesions in clinically normal skin are unable to withstand suction. The biochemical consequences of mechanical forces are frequently ignored but have been reviewed by Ryan.[23] Distortion or stretch normally stimulate tight adhesion by inhibiting proteases. It is possible in Hailey–Hailey disease that a failure in these mechanisms results in lysis of the fibrils of the cytoskeleton or membrane attachments when the skin is under tension.

In conclusion, we have demonstrated that there is a widespread intrinsic weakness in keratinocyte adhesion in Hailey–Hailey disease. The presence of intraepidermal cleavage in suction blisters induced on clinically normal skin may differentiate patients with Hailey–Hailey disease from those with bullous Darier's disease.

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References

1 Pye RJ. Benign familial chronic pemphigus (Hailey–Hailey disease) In: *Textbook of Dermatology*. (Rook AJ, Wilkinson DS, Ebling FJG, Champion RH, Burton JL, eds), 4th edn, Vol 2. Oxford: Blackwell Scientific Publications, 1986; 1629–31.
2 Lever WF, Schaumburg-Lever G. Familial benign pemphigus (Hailey–Hailey disease). In. *Histopathology of the Skin*. Philadelphia: J.B.Lippincott Co, 1983; 72–4.
3 Wilgram GF, Caulfield JB, Lever WF An electronmicroscopic study of acantholysis and dyskeratosis in Hailey–Hailey disease. *J Invest Dermatol* 1962; **39**: 373–81.
4 Gottlieb SK, Lutzner MA. Hailey–Hailey disease—an electron microscopic study. *J Invest Dermatol* 1970; **54**: 368–76.
5 Chorzelski T. Experimentally induced acantholysis in Hailey's benign pemphigus. *Dermatologica* 1962; **124**: 21–30
6 Kiistala U, Mustakallio KK. Dermoepidermal separation with suction. Electron microscopic and histochemical study of initial event of blistering on human skin. *J Invest Dermatol* 1967, **48**: 466–77.
7 Kobza Black A, Greaves MW, Hensby CN *et al.* A new method for recovery of exudates from normal and inflamed human skin. *Clin Exp Dermatol* 1977; **2**: 209–16.
8 Kiistala U, Mustakallio K *In-vivo* separation of epidermis by production of suction blisters. *Lancet* 1964; **i**: 1444–5.
9 Burge SM, Millard PR, Wojnarowska F, Ryan TJ. Darier's disease, a focal abnormality of cell adhesion. *J Cutan Pathol* 1990; **17**: 160–4.
10 Niordson AM, Sylvest B. Bullous dyskeratosis follicularis and acrokeratosis verruciformis. *Arch Dermatol* 1965; **92**: 166–8
11 Ganor S, Sagher F. Keratosis follicularis (Darier) and familial benign chronic pemphigus (Hailey–Hailey) in the same patient. *Br J Dermatol* 1965; **77**: 24–29.
12 O'Keefe EJ, Briggaman RA, Herman B. Calcium-induced assembly of adherens junctions in keratinocytes. *J Cell Biol* 1987; **105**: 807–17.
13 Green KJ, Geiger B, Jones JCR *et al.* The relationship between intermediate filaments and microfilaments before and during the formation of desmosomes and adherens-type junctions in mouse epidermal keratinocytes. *J Cell Biol* 1987, **104**: 1389–1402.
14 Takeichi M Cadherins: a molecular family essential for selective cell-cell adhesion and animal morphogenesis. *TIG* 1987; **3**: 213–17.
15 Burge SM, Garrod DR. An immunohistological study of desmosomes in Darier's disease and Hailey–Hailey disease. *Br J Dermatol* 1991; **124**: 242–51
16 Inohara S, Tatsumi Y, Tanka Y, Sagami S. Immunohistochemical localisation of desmosomal and cytoskeletal proteins in the epidermis of healthy individuals and patients with Hailey–Hailey disease. *Acta Derm Venereol (Stockh)* 1990; **70**: 239–41.
17 Hebert CA, Baker JB. Linkage of extracellular plasminogen activator to the fibroblast cytoskeleton: Colocalisation of cell surface urokinase with vinculin. *J Cell Biol* 1988; **106**: 1241–7.
18 Skerrow CJ. Plasmin induces pemphigus-type acantholysis in normal skin. *Br J Dermatol* 1986; **113**: 765.
19 Hunziker T, Vassali J-D. Plasmin induces acantholysis in skin organ cultures. *Arch Dermatol Res* 1987; **279**: 341–6.
20 Morioka S, Lazarus GS, Jensen PJ. Involvement of urokinase-type plasminogen activator in acantholysis induced by pemphigus IgG. *J Invest Dermatol* 1987; **89**: 474–7.
21 Isseroff RR, Rifkin DB. Plasminogen is present in the basal layer of the epidermis. *J Invest Dermatol* 1983, **80**: 297–9.
22 Burge SM, Ryan TJ, Cederholm-Williams SA. Darier's disease: an immunohistochemical study using antibodies to proteases. *Br J Dermatol* 1989; **121**: 613–21.
23 Ryan TJ. Biochemical consequences of mechanical forces generated by distension and distortion. *J Am Acad Dermatol* 1989, **21**: 115–130.

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Increase of lipid fluidity and suppression of proliferation resulting from liposome uptake by human keratinocytes *in vitro*

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Summary

The *in vitro* effects of liposomes on HaCaT human keratinocytes were studied with regard to their uptake, lipid fluidity and proliferation of the cells. Oligolamellar liposomes, prepared from soya bean phospholipids, had a mean size of 150 nm and consisted predominantly of phosphatidylcholine (83%) and phosphatidylethanolamine (10%) and the fatty acids comprised mainly linoleic acid (66%) or other unsaturated fatty acids. After 6 and 24 h of incubation with 1 and 0·1% w/v of liposomal lipids, phase-contrast microscopy revealed marked cytoplasmic vacuolization of the cells. Keratinocytes treated with the liposomes contained aggregations of multilaminated lipid material without delimiting cell membranes. The cellular lipid fluidity (reciprocal of diphenylhexatriene fluorescence polarization P-value) correlated with liposomal concentration and incubation time. A significant elevation of lipid fluidity ($P<0{\cdot}05$) was observed with 1 and 0·1% liposomes after 1 h of incubation (81·8±4·7 and 95·7±1·2% of control P value) and for 0·01% liposomes after 3 h (96·2±1·5%). Maximum fluidity occurred after 48 h of exposure to 1% liposomes (42·1±3·1%). Exposure to liposomal lipids for 24 and 48 h resulted in suppressed cell proliferation with 50% inhibition concentrations (IC_{50}), being 0·06% for incorporation of [^{3}H]-thymidine, 0·08% for [^{14}C]-amino-acid incorporation and >1% for protein content per well after 24 h of exposure. The cells were able to proliferate and lipid fluidity returned to normal within 7 days following discontinuation of incubation with liposomal lipids.

There is interest in the use of liposomes in topical cutaneous therapy.[1,2] Liposomes are globular microvesicles that consist of concentric amphiphilic lipid bilayers separated by hydrophilic compartments.[3] The liposomes are able to act as drug carriers and penetration enhancers through the fluidization of stratum corneum lipids as well as to localize drugs and prevent systemic resorption.[1,2,4–6] It is uncertain as to whether liposomes are able to penetrate through the barrier of the horny layer as intact vesicles to epidermal cells.[7] Substantial permeation of lecithin through the epidermis from topically applied liposomes was recently found to require a time of 5 h.[8] However, under certain circumstances topically applied liposomes may be able to come into direct contact with keratinocytes in the deeper part of the epidermis where there is a defective or incomplete stratum corneum, as in superficial wounds, some dermatoses and on mucocutaneous surfaces. We have studied as a model for direct cell targeting the effect of liposomal preparations on cultured human keratinocytes.

Methods

Liposomes

Natipide 08010A as a dispersion of 10% (w/v) liposomal soya bean phospholipids in 154 mM aqueous NaCl was supplied by A. Nattermann and Cie GmbH, Group Rhône–Poulenc, Cologne, Germany. The lipids consisted of 79–86% phosphatidylcholine, 10% phosphatidylethanolamine and 4% lysolecithin. The fatty acids comprised mainly the unsaturated linoleic acid (C18:2) (61–71%) with 10–15% palmitic acid (C16), 6–13% oleic acid (C18:1), 4–7% linolenic acid (C18:3), and 2–4% stearic acid (C18). The oligolamellar liposomes averaged 150±30 nm, as determined by the laser scattering technique, and were stored at −15°C.[9] Some experiments were performed with preparations of

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Natipide 08010A which had been supplemented with 0·2% liposomal Na-fluorescein (Merck, 61 Darmstadt, Germany). For incubation with keratinocytes the liposomal solutions were added to the complete culture medium, including serum which was subsequently filtered through 0·45-μm pores (Schleicher & Schüll, FP030/2, 3354 Dassel, Germany) giving final concentrations of liposomal lipid of 1·0, 0·5, 0·25, 0·1 and 0·01% (w/v).

Cell culture

HaCaT cells, an aneuploid human keratinocyte cell line with normal keratinization established from normal human adult skin,[10] were grown in Dulbecco's modified Eagle's medium (DMEM) containing 1% antibiotic–antimycotic solution (Gibco Cat. No. 043–5240H), 4 mM L-glutamine (Gibco Cat. No. 043-5030H) and 10% fetal calf serum (FCS, Gibco Cat. No. 011–6290H). The humidified atmosphere was kept at 10% CO_2 and 37°C.

For growth assay cells were seeded at a density of 4×10^4 cells per 1·8 cm^2 area (plastic 24-well dishes, Greiner). Two days after plating subconfluent cultures were incubated with 1·0, 0·5, 0·25, 0·1 and 0·01% liposomes for 24 and 48 h and prepared for measurement. In another set of experiments 1×10^4 cells were plated per dish, exposed to 0·1% liposomes during day 2 and 3 and subsequently grown in standard medium until day 14. Parallel experiments were performed in order to determine the number of attached cells by means of a Neubauer haemocytometer and their viability by trypan blue exclusion test.[11]

Cellular lipid fluidity was studied in cultures which had been grown to confluence in 75-cm^2 flasks (Falcon). The confluent cultures were incubated with 1·0, 0·1 and 0·01% liposomes for 1, 3, 6, 12, 24 and 48 h and processed for lipid fluidity measurement. Some cultures were subjected to an 11-day observation period under standard conditions after an initial exposure to 0·1% liposomes for 2 days. All experiments included controls incubated with physiological saline (154 mM aqueous NaCl) added in appropriate concentrations to the culture medium.

Microscopy

Phase-contrast microscopy (Olympus IMT-2) was used for the routine monitoring of cultures. Experiments employing 0·1% liposomes loaded with fluorescein were performed on HaCaT cells which had been cultured in RPMI 1640 without phenol red (Gibco Cat. No. 041–0240411) including FCS, antibiotics and antimycotic as well as L-glutamine (5% CO_2). This procedure was chosen in order to rule out strong fluorescence in the background. For fluorescence microscopy (Leitz Dialux 20 EB, I2/3 filter combination) cells were grown on glass cover slips which then were reverse-mounted on a glass slide.

For electron microscopy (EM10, Zeiss) confluent HaCaT cells in plastic dishes (3·5 cm in diameter) were treated with liposomes, fixed with 3% glutaraldehyde, with and without 1% tannin (Merck, Cat. No. 773), dehydrated in rising concentrations of ethanol and embedded in Durcupan ACM (Fluka, CH-Basel). Ultrathin sections were stained with uranyl acetate and lead citrate.

Cellular uptake of [^{3}H]-thymidine and [^{14}C]-amino acid

After incubation with liposomes cultures were pulse labelled (2 h) simultaneously with 1 μCi methyl-[^{3}H]-thymidine (specific activity 77·9 Ci/mmol; NEN DuPont, 638 Bad Homburg, Germany, NET-027Z) and 0·5 μCi [^{14}C]-amino acids (mixture of 15 different amino acids, specific activity 1·85 GBq/milligramatom of carbon; Amersham, 33 Braunschweig, Germany, CFB.104) per ml DMEM and culture well. Attached cells were exposed to 0·05% trypsin/0·02% EDTA (Gibco Cat. No. 043-5300M) at 37°C for 45 min. After precipitation by ice-cold 5% trichloroacetic acid the cells were harvested for the Rackbeta scintillation counter (LKB, two channel mode). The incorporation of [^{3}H]-thymidine and [^{14}C]-amino acids served as measures for the DNA and protein synthesis of the cultures.[12,13]

Measurement of cellular protein

Protein content of attached cells was measured by a modified Bradford method as reported previously.[13,14] In brief, the cultures were rinsed twice in methanol, then 0·5 N NaOH was added and the wells were gently agitated at room temperature for 16 h. The probes were treated with BioRad reagent (Cat. No. 500–0006) and measured in a calculator-equipped photometer at 595 nm using bovine serum albumin as standard.

Fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH)

The culture supernatant was discarded and the cells were rinsed with phosphate-buffered saline (PBS). After trypsinization the cell suspension was washed three times by centrifugation at 80 *g* for 10 min, diluted to a concentration of 2×10^6 cells/ml PBS and incubated with an equal volume of a 2 μM microdispersion of lipophilic DPH fluorophore (Fluka, CH-Buchs, Cat. No. 43050) in PBS for 60 min at 37°C.[15–17] After centrifugation the DPH-labelled cells were resuspended in PBS while the extinction (450 nm[18]) of the cell suspension was adjusted to 0·55 in order to keep the depolarizing stray-light effect constant.[15] This is known to correlate positively with the cell density.[18] The degree of fluorescence polarization, the P-value, was measured in a microviscosimeter MV-1a (Elscint Company, Haifa, Israel) at 25°C. The P-value is defined as $P=(I_{pa}-I_{pe})/(I_{pa}+I_{pe})$ with fluorescence intensities (I) which are detected in parallel (pa) or perpendicular (pe) orientation to the electric vector of the excitation light. Lipid fluidity is known to correlate negatively with the P-value, reaching an upper limit of 0·5 in case of extreme rigidity.[16,17,19] For the determination of the fluidity of pure liposomes, they were diluted with the 2 μM DPH microdispersion until an extinction of 0·55 was acheived (450 nm). After the liposomal suspension had been incubated at 37°C for 60 min its P-value was measured at 25°C.

Statistical analysis

Each treatment comprised at least six cultures. Statistical comparisons were performed using the Mann–Whitney *U*-test.[20] Multiple comparisons required Bonferroni's adjustment.[21] The overall level of significance was set to $P<0{\cdot}05$.

Results

Morphology

After incubation with liposomal lipids, phase-contrast microscopy showed cyclic and polycyclic homogeneous cytoplasmic vacuoles, mostly close to the nuclei (Fig. 1). Rinsing the cultures with methanol did not remove these structures, which ruled out lipid droplets adhering to the outer cell surface. The number and size of the vacuoles correlated directly with the concentration of liposomes and the time of incubation. Cytoplasmic vacuolization was marked after incubation with 1·0 and 0·1% liposomes for 6 and 24 h, respectively, and was scarcely detectable with 0·01% liposomes even after 48 h of incubation. In control cultures vacuolizations were extremely rare. Incubation with fluorescein-labelled liposomes led to a homogeneous fluorescence inside the multiple cyclic cytoplasmic structures (Fig. 2). When incubation was started at a subconfluence, incubation with ≥0·25% liposomes for 48 h resulted in a reduction of the density of attached cells and an increase of the cell size compared to the control (Fig. 1). However, signs of direct cytotoxicity such as cell detachment or deformation were not seen during the 48-h liposomal treatment.

Ultrastructurally, clusters of 'empty' vacuoles surrounding the nuclei were typically seen after incubation with 1% liposomes for 24 h. Tannin impregnation revealed single oligolamellar bodies as well as huge multilamellar aggregrates inside the cytoplasm which were absent in the controls (Fig. 3).

Alterations in lipid fluidity

The degree of DPH fluorescence polarization of the pure liposome solution (P-value = 0·090 ± 0·004) was very low in comparison to untreated keratinocytes (P-value = 0·259 ± 0·005). Thus, the liposomes were much more fluid than the cellular lipids. When keratinocytes were exposed to liposomes, the cellular lipid fluidity steadily increased with the increase of liposome concentration and incubation time (Fig. 4). A significant fluidization was evident for 0·1 and 1·0 liposomes after 1 h incubation and for 0·01% after 3 h. The maximum fluidization of cell lipids was reached after 48 h of exposure with 1·0% liposomes (P-value = 0·109 ± 0·008). This was very close to the intrinsic P-value of the pure liposome preparation.

The reduction of the cellular P-value to two-thirds of the control level after incubation with 0·1% liposomes for 48 h persisted under subsequent standard culture without liposomes for at least 4 days. The P-value first returned to the control level at day 7 and maintained this level until day 11 after the discontinuation of liposomal treatment.

Keratinocyte proliferation and viability

As compared to the control values, which were set at 100%, exposure of keratinocytes to 0·01 and 0·1% liposomes resulted in [^{3}H]-thymidine incorporation of 74·6 ± 5·1 and 31·3 ± 4·9%, respectively, after a 24-h incubation period, and of 88·5 ± 9·9 and 18·9 ± 3·5% after 48 h (Fig. 5a). The incorporation of [^{14}C]-amino acid was 84·9 ± 5·2 and 40·9 ± 4·7% after 24 h, and 89·5 ± 9·2 and 23·8 ± 4·6% after 48 h (Fig. 5b). The uptake of [^{3}H]-thymidine and [^{14}C]-amino acid uptake were significantly ($P<0{\cdot}05$) suppressed by liposomal concentrations of ≥0·01% after 24 h and ≥0·1% after 48 h. A 50% inhibition concentration (IC_{50}) of 0·06% liposomal lipids was calculated for DNA synthesis after

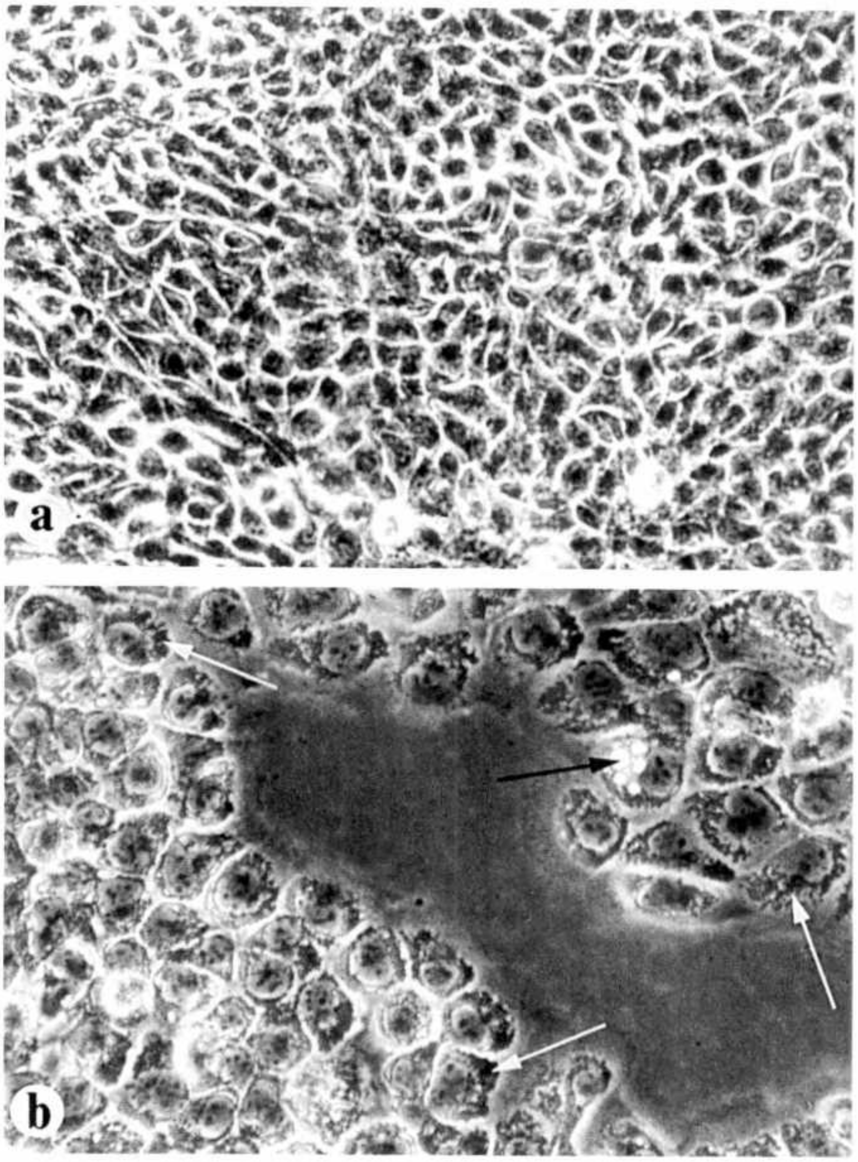

Figure 1. HaCaT keratinocytes incubated for 48 h with (a) 1% physiological saline (control), (b) 1% liposomes, showing reduced cell density, increased cell size and cytoplasmic vacuoles. Small (primary) vacuoles (white arrows) were found in nearly all cells; only a few large (secondary) vacuoles (black arrow) were found (phase contrast, × 280).

24 and 48 h as well as for protein synthesis after 48 h. With a 24-h incubation period the IC_{50} for the latter parameter was 0·08%.

Liposomal concentrations of 0·01 and 0·1% gave total protein contents of 90·6 ± 11·0% and 87·3 ± 10·5%, respectively, after 24 h, and of 107·4 ± 13·9 and 66·7 ± 9·6% after 48 h (Fig. 5c). The effects were significant ($P < 0{\cdot}05$) only with liposomal concentrations of ≥ 0·1% after 24 h and 48 h. Corresponding IC_{50} values were greater than 1·0%.

A significant decrease in the number of attached cells per culture well became evident after incubation for 48 h with ⩾ 0·25% liposomes (Table 1). Cell viability remained unaffected at all liposomal concentrations tested during the 48-h incubations (Table 1).

Monitoring of the mean total protein content per well at 4, 7 and 11 days after an initial incubation for 48 h with 0·1% liposomes, showed a slow cell growth by a factor of 1·2, 1·8 and 2·6 as compared to the protein value which was determined immediately after the exposure. The control values exceeded those of liposome-treated cultures by a factor of 4·6, 4·3 and 3·0 at 4, 7 and 11 days, respectively. At day 4 the cell viability of the attached cells dropped significantly ($P < 0{\cdot}05$) to 65·4 ± 16·9% and had returned to the average control level of 95–99% by day 7.

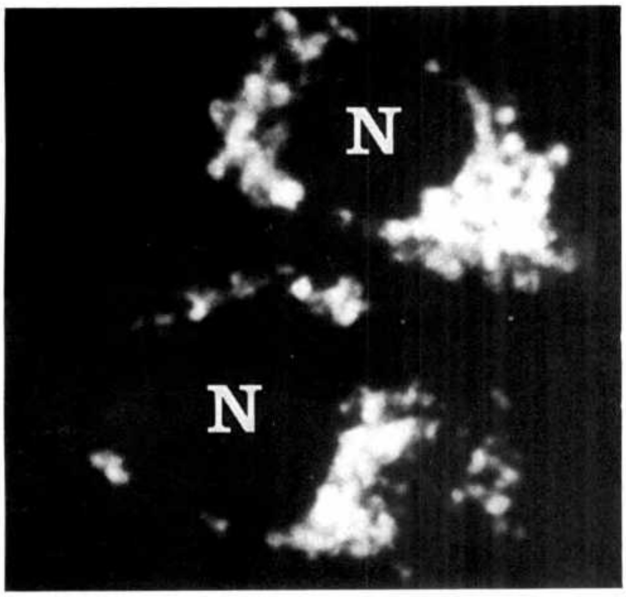

Figure 2. Fluorescence microscopy of two HaCaT cells after 48 h exposure to 0·1% liposomes loaded with fluorescein (× 1200). Multiple cyclic areas of fluorescence inside the cytoplasm surrounded the dark nuclear areas (N).

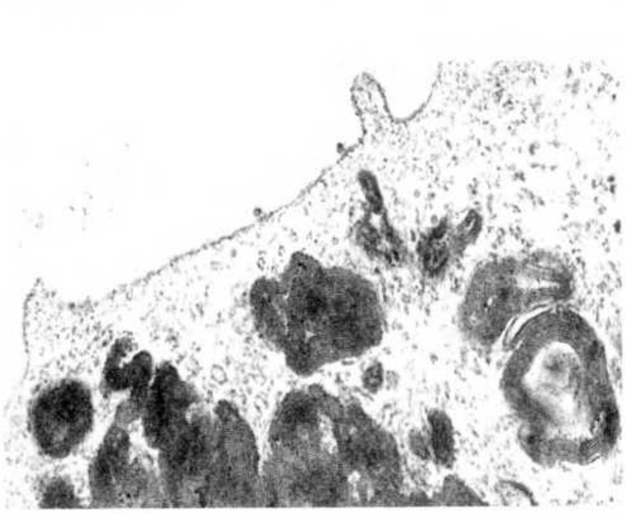

Figure 3. Ultrastructure of a HaCaT keratinocyte after 24 h incubation with 1% liposomes (1% tannin impregnation, × 89,800). There were no cell membranes around the laminated lipid aggregates.

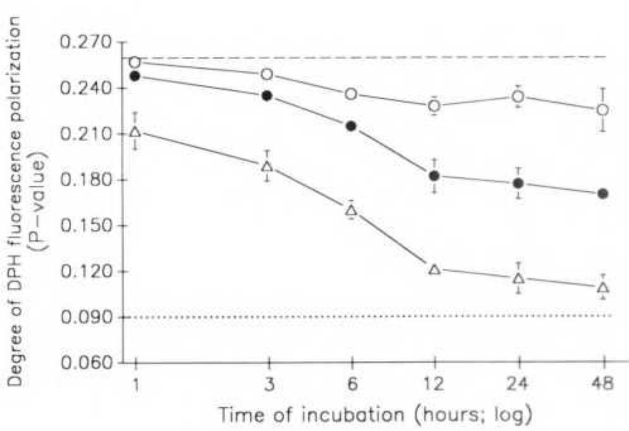

Figure 4. Fluidization of HaCaT cell lipids as a function of liposomal concentration and incubation time. The degree of 1,6-diphenyl-1,3,5-hexatriene (DPH) fluorescence polarization (syn. P-value) served as a reciprocal measure for the cellular lipid fluidity. Average lipid fluidities of control cells without liposomal treatment and of pure liposomes are indicated by dashed and dotted lines, respectively. Exposure for 48 h with 0·01% (○), 0·1% (●) and 1·0% (△) liposomes significantly ($P<0{\cdot}05$) reduced the P-value to 86·9 ± 5·4, 65·6 ± 1·9 and 42·1 ± 3·1% in comparison to the controls (100%).

Discussion

The interactions between liposomes and keratinocytes *in vitro* have been described for non-human systems.[22,23] This present study demonstrates that human keratinocytes are able to incorporate considerable amounts of liposomes. This was evident from the dose- and time-dependent formation of cytoplasmic vacuoles that contained multilaminated material as well as liposome-derived fluorescein. Cultures of guinea-pig epidermal cells incubated with distearoylphosphatidylcholine-dicetylphosphate liposomes enclosing hydrophilic 6-carboxyfluorescein showed granular cytoplasmic fluorescence with a centripetal spreading within the cells after 1 h of exposure.[22] The uptake of liposomes was also demonstrated by the marked fluidity of cellular lipids during the incubation of the cells. Human keratinocytes, like those of the guinea-pig,[22] internalized liposomes at a fast rate with a significant decrease of the P-value after only 1 h of incubation.

The mechanism of liposomal incorporation is thought to be by stable adsorption with lipid transfer, membrane fusion or phagocytosis.[24–27] Keratinocytes are known to phagocytose various sized particles including *Candida albicans*, Thorotrast, melanin, charcoal, erythrocytes, Langerhans cell debris, fibrin fragments and latex beads.[28] However, in our study a membrane delimiting the lipid vacuoles was not visible and we observed free lipid accumulation in the cytoplasm, as described for adipocytes, and not within endosomes or lysosomes.[29] Our findings support an uptake of the liposomes through membrane fusion. The factors that are known to promote such a fusion, high fluidity of the lipid vesicles and an elevated temperature,[25,26] were present in our system. A secondary leakage of the liposomal lipids into the cytoplasm following their initial uptake within small endosomes is less likely.

The increased spread of the cells caused by liposomes could be due to an increased cellular volume and also a

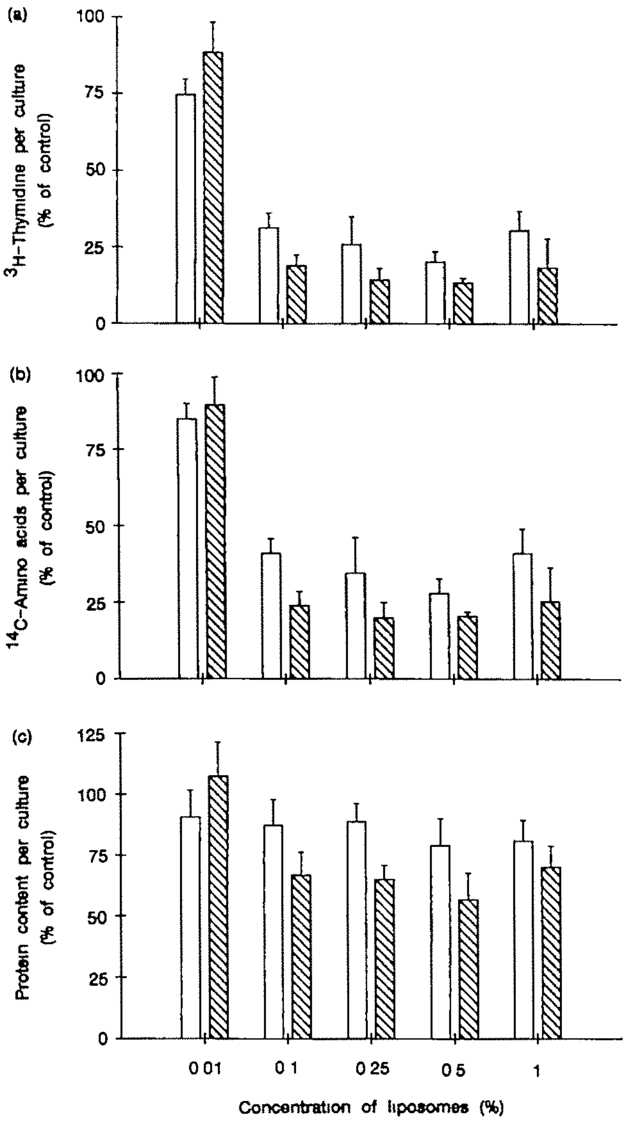

Figure 5. Significant decreases ($P<0{\cdot}05$) in (a) DNA synthesis and (b) protein synthesis per HaCaT cell culture under liposomal concentrations of ≥0·01% (after 24 h exposure) and ≥0·1% (48 h). (c) A significant decrease in total protein content of attached cells was observed at concentrations ≥0·1% (24 h and 48 h). □, 24 h; ■, 48 h.

decrease of cell density because of the inhibition of proliferation. It may also be due to increased lipid fluidity. The most sensitive functional parameter for the influence of liposomes, as indicated by the IC_{50} values, was the inhibition of DNA synthesis (0·06% liposomes after 24 h incubation), followed by protein synthesis (0·08%) and finally by protein content (>1·0%). Even though there was a marked vacuolization of the cells during 48 h liposomal treatment, there was no direct cytotoxic effect, as indicated by unimpaired cell viability and a normal rate of cell shedding. However a transient decrease of viability was observed 4 days following discontinuation of exposure to the liposomes. At day 7 the cell viability had normalized and this was accompanied by a steady growth. In accordance with our findings only negligible immediate effects on the viability of guinea-pig keratinocytes were found after 4 h of treatment with 0·2% soya bean phospholipid liposomes.[23] Subcultured normal human keratinocytes, less proliferative than HaCaT cells and primarily grown on lethally irradiated 3T3 fibroblasts with supplementation of growth factors, responded to a lesser degree to the growth-inhibitory effects of liposomes (data not shown). Substantial toxic alterations of guinea-pig epidermal cells have been observed after exposure for 72 h to 0·1% liposomal lipids prepared from egg lecithin,[22] for which the high content of saturated fatty acids in egg lecithin may be responsible.

Recently the degree of DPH-fluorescence polarization was measured in suspension cultures of guinea-pig keratinocytes with liposomes.[23] There was a decrease in the P-value by 54% as a result of 4 h of treatment with 0·2% soya bean phospholipid liposomes. The fluidizing effect, which was accompanied by reduced concanavalin A binding, was more pronounced as compared to our data. This may be explained by the liposomal composition (30% phosphatidylcholine, 35% phosphatidylinositol and 35% phosphatidylethanolamine) used by

Table 1. Counts of attached cells per well and their viability after incubation for 48 h of HaCaT keratinocyte cultures with medium supplemented with liposomes or physiological saline (control). The cell number per well was 97,917±14,201 immediately before the onset of the incubation

Per cent liposomal lipids	Cell number (means±SD)		Cell viability (means±SD)	
	Treatment	Control	Treatment	Control
0·01	552,292±21,915	538,958±36,024	97·0±1·3	97·0±1·5
0·1	521,875±40,634	530,000±56,347	95·4±2·8	97 1±1·7
0·25	402,500±53,921*	470,208±47,351	96·3±3·0	95·6±2·5
0 5	342,500±26,363*	461,667±56,941	94·7±3·9	97·2±2·0
1 0	357,917±60,118*	490,417±32,631	94·5±2 1	93·5±2·6

*$P<0{\cdot}05$.

Callaghan *et al.*[23] and by the fact that liposomes were added to cell suspensions allowing a better liposomal access to the cells over their whole surface.

The present finding that liposomal material greatly accumulated inside the cells and the fact that DPH is incorporated in all intracellular lipids[18,30,31] should give rise to special caution in interpreting the P-values of liposomal-treated keratinocytes solely in terms of plasma membrane fluidity. Nevertheless, the P-value seems an excellent parameter for monitoring liposomal incorporation by keratinocytes. Moreover the restoration of the P-value by day 7 after discontinuation of liposomal exposure reflected the degradation of liposomal lipids.

Our data and those from the literature[23] demonstrating marked fluidization of keratinocytes by phospholipid liposomes are further evidence of a positive correlation between cellular phospholipid/cholesterol ratio and (membrane) lipid fluidity.[19,32,33] This is supported by previous studies showing that keratinocyte lipid fluidity is down-regulated *in vitro* by the cellular uptake of cholesterol-hemisuccinate and -sulphate[34] and by the induction of differentiation through the elevation of extracellular calcium,[35] and *in situ* during differentiation[36,37] accompanied by a rising plasma membrane cholesterol content.[38] These indications of the modulation of cell membrane fluidity are of functional importance because this is known to affect in a complex manner the lateral and rotational mobility of protein determinants as well as their degree of exposure (vertical phase separation).[32]

In conclusion, with regard to the future topical application of liposomal-entrapped drugs, the present results point to a specific effect of the liposomes themselves. When incorporated by human keratinocytes liposomes increased cellular lipid fluidity, possibly regulating cell surface receptor functions and slowing down cell proliferation.

Acknowledgments

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References

1 Handjani-Vila RM, Guesnet J. Les 'liposomes': un avenir prometteur en dermatologie. *Ann Dermatol Venereol* 1989, **116**: 423–30.
2 Schafer-Korting M, Korting HC, Braun-Falco O. Liposome preparations: A step forward in topical drug therapy for skin disease? A review *J Am Acad Dermatol* 1989; **21**: 1271–5.
3 Bangham AD. Liposomes: an historical perspective. In: *Liposomes* (Ostro MJ, ed). New York: Marcel Dekker, 1983, 1–26.
4 Mezei M. Liposomes in the topical application of drugs: a review. In: *Liposomes as Drug Carriers* (Gregoriadis G, ed). Chichester: Wiley, 1988; 663–78.
5 Patel HM. Liposomes as a controlled-release system. *Biochem Soc Trans* 1985; **13**: 513–6.
6 Siciliano AA. Topical liposomes—an update and review of uses and production methods. *Cosmet Toilet* 1985; **100**: 43–6.
7 Landmann L. The epidermal permeability barrier. *Anat Embryol* 1988; **178**: 1–13.
8 Wohlrab W, Lachmann U, Lasch J. Penetration of lecithin from hydrocortisone-containing liposomes into human skin. *Dermatol Monatssch* 1989; **175**: 344–7.
9 Lautenschläger H, Roding J, Ghyczy M. Über die Verwendung von Liposomen aus Soja-Phospholipiden in der Kosmetik. *Seifen-Öle-Fette-Wachse* 1988; **114**: 531–4.
10 Boukamp P, Petrussevska RT, Breitkreutz D *et al.* Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J Cell Biol* 1988; **106**: 761–71.
11 Fallon HJ, Frei E, Davidson JD *et al.* Leukocyte preparations from human blood: evaluation of their morphologic and metabolic state. *J Lab Clin Med* 1962; **59**: 779–91.
12 Klem EB. Effects of antipsoriasis drugs and metabolic inhibitors on the growth of epidermal cells in culture. *J Invest Dermatol* 1978; **70**: 27–32.
13 Reichert U, Jacques Y, Grangeret M, Schmidt R. Antirespiratory and antiproliferative activity of anthralin in cultured human keratinocytes. *J Invest Dermatol* 1985; **84**: 130–4.
14 Bonnekoh B, Wevers A, Jugert F *et al.* Colorimetric growth assay for epidermal cell cultures by their crystal violet binding capacity. *Arch Dermatol Res* 1989; **281**: 487–90.
15 Bonnekoh B, Thiele B, Krueger GRF, Mahrle G. Lipid fluidity of human and guinea-pig epidermal cells: temperature dependence in comparison to nonepidermal cells. *Arch Dermatol Res* 1987; **279**: 278–80.
16 Inbar M, Shinitzky M, Sachs L. Microviscosity in the surface membrane lipid layer of intact normal lymphocytes and leukemic cells. *FEBS Lett* 1974; **38**: 268–70.
17 Shinitzky M, Inbar M. Difference in microviscosity induced by different cholesterol levels in the surface membrane lipid layer of normal lymphocytes and malignant lymphoma cells. *J Mol Biol* 1974; **85**: 603–15.
18 Johnson SM, Nicolau C. The distribution of 1,6-diphenyl-hexatriene fluorescence in normal human lymphocytes *Biochem Biophys Res Commun* 1977; **76**: 869–74.
19 Shinitzky M, Yuli I. Lipid fluidity at the submacroscopic level: determination by fluorescence polarization. *Chem Phys Lipids* 1982; **30**: 261–82.
20 Mann HB, Whitney DR. On a test of whether one of two random variables is stochastically larger than the other. *Ann Math Statist* 1947; **18**: 50–60.
21 Fleiss JL. *The Design and Analysis of Clinical Experiments*. New York: Wiley, 1986; 105.
22 Bertschi W. Herstellung von Liposomen aus synthetischen Lipiden und Einschluss verschiedener Modellsubstanzen: Toxizitats prufungen im Hinblick auf eine mögliche topische Applikation. *PhD Thesis*, Naturwissenschaftliche Fakultät ETH, Zurich, 1982.
23 Callaghan TM, Metezeau P, Gachelin H *et al.* Modulation of the binding and endocytosis of concanavalin A by guinea pig keratinocytes: reversible antagonistic effects of cholesterol and phospholipid-liposomes. *J Invest Dermatol* 1990; **94**: 58–64.
24 Huang L, Pagano RE. Interaction of phospholipid vesicles with cultured mammalian cells. I. Characteristics of uptake. *J Cell Biol* 1975; **67**: 38–48.

25 Machy P, Lesermann L. *Liposomes in Cell Biology and Pharmacology—3. Interactions Between Cells and Liposomes*. London. Libbey, 1987; 99–165.

26 Pagano RE, Huang L. Interaction of phospholipid vesicles with cultured mammalian cells. II. Studies of mechanism. *J Cell Biol* 1975; **67**: 49–60.

27 Pagano RE, Weinstein JN. Interactions of liposomes with mammalian cells. *Ann Rev Biophys Bioeng* 1978; **7**: 435–68.

28 Csato M, Bozoky B, Hunyadi J, Dobozy A. Candida albicans phagocytosis by separated human epidermal cells. *Arch Dermatol Res* 1986; **279**: 136–9.

29 Breathnach AS. *The Ultrastructure of Human Skin*. London: JA Churchill, 1971.

30 Collard JG, DeWildt A. Localization of the lipid probe 1,6-diphenyl-1,3,5-hexatriene (DPH) in intact cells by fluorescence microscopy. *Exp Cell Res* 1978; **116**: 447–50.

31 Van Hoeven RP, Van Blitterswijk WJ, Emmelot P. Fluorescence polarization measurements on normal and tumour cells and their corresponding plasma membranes. *Biochim Biophys Acta (Amst)* 1979; **551**: 44–54.

32 Muller CP, Krueger GRF. Modulation of membrane proteins by vertical phase separation and membrane lipid fluidity. Basis for a new approach to tumor immunotherapy. *Anticanc Res* 1986; **6**: 1181–94

33 Yeagle PL. Lipid regulation of cell membrane structure and function. *FASEB J* 1989; **3**: 1833–42

34 Bonnekoh B, Thiele B, Daefler S *et al.* X-linked ichthyosis model: epidermal cell lipid modulation by cholesterol sulfate as measured by fluorescence polarization. *J Invest Dermatol* 1988; **90**: 238.

35 Ponec M, Tertoolen L, Kempenaar J, de Laat SW. Lateral mobility of plasma membrane lipids and lipid synthesis during differentiation of cultured keratinocytes. *J Invest Dermatol* 1986, **87**: 162.

36 Tanaka T, Hidaka T, Ogura R, Sugiyama M. Changes of membrane fluidity and Na$^+$, K$^+$-ATPase activity during cellular differentiation in the guinea pig epidermis *Arch Dermatol Res* 1988; **280**: 29–32.

37 Tanaka T, Sakanashi T, Kaneko N, Ogura R. Spin labelling study on membrane fluidity of epidermal cell (cow snout epidermis). *J Invest Dermatol* 1986; **87**: 745–7.

38 Gray GM, King IA, Yardley HJ. The plasma membrane of Malpighian cells from pig epidermis: isolation and lipid and protein composition. *Br J Dermatol* 1980; **103**: 505–15.

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Regulation of β_2-adrenergic receptors in keratinocytes: glucocorticoids increase steady-state levels of receptor mRNA in foetal rat keratinizing epidermal cells (FRSK cells)

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Summary

Glucocorticoids increase the β-adrenergic adenylate cyclase response of epidermal keratinocytes. Using FRSK cells, a cultured cell line of foetal rat keratinocytes, the regulatory mechanism of the β-adrenergic augmentation effect was investigated. Treatment with dexamethasone (1×10^{-6} M) increased by 1·5-fold the β-adrenergic adenylate cyclase response of FRSK cells. The effect was observed at 6 h incubation and remained for at least 48 h. The prostaglandin E–adenylate cyclase response was also increased 1·5-fold by glucocorticoid treatment. Neither the adenosine–adenylate cyclase response nor cholera toxin- or forskolin-induced cyclic AMP accumulations were altered. Northern blot hybridization showed that levels of the β_2-adrenergic receptor mRNA increased within 3 h, while actin-, Gs-α, Gi-2α, Gi-3α mRNA levels were unchanged. Testosterone, 17β-oestradiol, and progesterone had no effect on either the β_2-adrenergic adenylate cyclase response or the expression of β_2-adrenergic receptor mRNA. The increase in the numbers of the β-adrenergic receptors was visualized by immunofluorescence with an antibody specific for the β_2-adrenergic receptor. Our results indicate that glucocorticoids regulate the β_2-adrenergic adenylate cyclase response of FRSK cells through the enhanced expression of the receptor.

It has been reported that epidermal keratinocytes are regulated by glucocorticoids resulting in the augmentation of the β-adrenergic adenylate cyclase response.[1,2] In culture, this β-adrenergic augmentation effect is detected after 4–6 h, and is inhibited by various protein synthesis inhibitors.[1,3] These findings suggest that glucocorticoids act through the synthesis of specific steroid-inducible proteins, which may include lipocortin, an endogenous phospholipase A_2 inhibitor protein.[4–6]

Besides possible regulation by lipocortin in various cell systems glucocorticoids increase the β-adrenergic receptor through the increased transcription of the receptor mRNA.[7–9] The β_2-adrenergic receptor gene has at least four consensus glucocorticoid responsive elements in 5′ flanking, coding, as well as 3′ flanking regions.[10–12]

The β-adrenergic receptor in keratinocytes is the β_2 type.[13,14] Thus it is possible that glucocorticoids regulate the expression of the β_2-adrenergic receptor gene in keratinocytes as in other cell systems. However, because of the weak expression of the steady-state β_2-adrenergic receptor mRNA in normal keratinocytes, evidence for the transcriptional control of β_2-adrenergic receptor has not been available.

FRSK cells are a cultured cell line derived from foetal rat keratinocytes.[15] Recently we found that the adenylate cyclase system of FRSK cells is very similar to that of normal human epidermis.[14] The cells possess β_2-adrenergic, prostaglandin E, and adenosine receptors, which activate the adenylate cyclase. They also possess Gs and Gi-2,3, which are affected by cholera toxin and islet-activating protein, respectively. We also found that FRSK cells were regulated by glucocorticoids resulting in β-adrenergic augmentation.

In contrast to the normal epidermis, FRSK cells express significant amounts of the β_2-adrenergic receptor mRNA.[14] Therefore, we investigated the nature of regulation by glucocorticoids in FRSK cells. Our results indicate that, besides possible regulation by lipocortin, glucocorticoids may augment the β-adrenergic adenylate cyclase response of keratinocytes through increased expression of the β_2-adrenergic receptor.

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Methods

Cell culture

FRSK cells, a cultured cell line of foetal rat epidermal keratinocytes,[15] were obtained from the Japanese Cancer Research Bank (JCRB) Cell, Foundation for Promotion of Cancer Research. The FRSK cells were cultured in Dulbecco's modified Eagle's medium supplemented with 5% foetal calf serum (FCS), 100 units/ml penicillin, and 100 μg/ml streptomycin at 37°C in 5% CO_2 in air, and maintained at subconfluence by subculture every 4–5 days. Cells were seeded at concentrations of $4{\cdot}0 \times 10^5$ cells/5 ml into plastic dishes of 50 mm diameter.

Steroid treatments

Cells were treated with 0·25% trypsin in 0·5% EDTA solution for 5 min at 20°C and seeded in a 24-well culture plate (16-mm diameter wells) at a concentration of 4×10^4 cells/well with 1 ml of fresh medium. They were cultured for 2 days by which time the cell density was 2×10^5 cells/ml. One millilitre of fresh culture medium containing various steroids were added to each well and incubations were performed for the indicated time. Steroids were dissolved in ethanol and the final concentration of ethanol was less than 0·5% v/v. Appropriate concentrations of ethanol were added to the control medium. These concentrations of ethanol had no effect on either adenylate cyclase responses nor on the expression of β_2-adrenergic receptor mRNA. At the indicated time of the incubation, cells were incubated for cyclic AMP accumulation or were collected for RNA extraction. Five per cent FCS and antibiotics were added to the culture medium throughout the steroid treatment.

Incubation for cyclic AMP accumulation

Following the steroid treatments, the β-adrenergic-, prostaglandin E-, histamine- and adenosine-adenylate cyclase responses were measured as described previously.[14,16] Concentrations of epinephrine, prostaglandin E_2, histamine and adenosine were 50 μM, 30 μM, 1 mM and 2 mM, respectively. These concentrations were sufficient for the maximal accumulation of cyclic AMP. Incubations were performed at 37°C for 5 min in the presence of 1 mM IBMX for the epinephrine, prostaglandin E_2 and histamine experiments, and 250 μM papaverine for the adenosine experiment.[16] Following the incubation, the reaction was stopped by removing the medium and adding 0·5 ml 5% trichloroacetic acid to extract the cyclic AMP.[16] Cyclic AMP was measured by radioimmunoassay using a Yamasa cyclic AMP assay kit (Yamasa Shoyu, Tokyo, Japan). Forskolin- and cholera toxin-induced cyclic AMP accumulations were performed as described previously.[14,17]

RNA extraction

Following the steroid treatment, keratinocytes (about 1×10^7 cells) were homogenized in guanidine thiocyanate solution and total RNA was extracted by the caesium guanidine method.[18] Poly(A)$^+$ RNA was purified on an oligo-(dT)-cellulose column.[18] RNA concentration was adjusted by measuring the optical density at 260 nm.

Northern blot analysis

Poly(A)$^+$ RNA (20 μg) was denatured with 1 M glyoxal, electrophoresed in 1% agarose gel in circulating 0·1 M phosphate buffer, and transferred to a nylon filter.[19] The filters were then baked at 80°C for 1 h.

Cloned probes

The human β_2-adrenergic receptor cDNA[20] was obtained from American Type Culture Collection (ATCC) (Rockville, MD, U.S.A.). The rat Gs-α, Gi-2α and Gi-3α cDNAs were gifts from Professor Y. Kaziro, Institute of Medical Science, University of Tokyo, Japan.[21,22] β-Actin cDNA was a gift from Dr F. Gunning, Stanford University School of Medicine and the Veterans Administration Medical Center.

Hybridization

Probes were labelled with α-[^{32}P]-dCTP (3000 Ci/mmol) by the random primer method to specific activities of 2–8×10^8 c.p.m./μg.[18] Hybridization was performed with random primed probes in 10% dextran sulphate, 1·5 × SSPE (0·23 M NaCl, 0·02 M NaH_2PO_4, 1·5 mM EDTA, pH 7·4), 1% $NaDoSO_4$ (sodium dodecylsulphate), 0·5% skimmed milk, and denatured salmon sperm DNA (500 μg/ml) at 65°C for 16–24 h. After hybridization, filters were washed in 2 × SSC (0·15 M NaCl, 0·015 M sodium citrate, pH 7·0), and 0·1 × SSC containing 0·1% $NaDoSO_4$ for 15 min each at 20°C and 0·1 × SSC containing 0·1% $NaDoSO_4$ at 65°C for 10 min until the radioactive bands appeared on a Geiger counter. The filters were then packed in plastic bags and exposed to X-ray films with intensifier screens at − 70°C for 1–2 days. After autoradiography, the previously hybridized probe was removed by incubating in 50% formamide/0·1% phosphate buffer for 1 h at 65°C and rinsing in 2 × SSC for 30 min at 20°C. Next they were sequentially hybridized to different radiolabelled probes.

Indirect immunofluorescence of β_2-adrenergic receptor

Indirect immunofluorescence of β_2-adrenergic receptor was performed with FRSK cells treated with 1 μM dexamethasone for 24 h. Cells were dissociated with trypsin-EDTA treatment (see above) and were adjusted to $1{\cdot}5 \times 10^6$ cells/ml in PBS, pH 7·2. The cells were then fixed with cold acetone on Hendley–Essex slides, washed with PBS, and were saturated with PBS-gelatin (0·3%) for 1 h at 20°C. The cells were probed with antireceptor antibody BRK-1 (IgM class-antibody)[23] at a dilution of 1:100 in PBS-gelatin for 1 h at 37°C. The cells were then washed with PBS-gelatin and incubated with rabbit anti-mouse serum IgM at a dilution of 1:100 for 1 h at 37°C. The cells were then washed with PBS-gelatin and incubated with fluorescein-conjugated goat anti-rabbit Ig for 1 h. The slides were washed with PBS-gelatin, and mounted with glycerol and observed under a fluorescent microscope. Photographs were taken with a Fujichrome RD 135-36. The antireceptor antibody was donated by Professor A.D.Strosberg (Institute Pasteur, Paris, France).

Materials

Dulbecco's modified Eagle's medium was purchased from Gibco (Grand Island, New York). Penicillin and streptomycin were obtained from M.A. Bioproducts (Walkersville, Maryland). [α-^{32}P]deoxy CTP was obtained from Amersham Japan (Tokyo, Japan). Dexamethasone, testosterone, 17-β oestradiol, progesterone, and cholera toxin were purchased from Sigma (St Louis, MO, U.S.A.). Forskolin 7-deacetyl-7-o-hemisuccinic acid was the product of Calbiochem (La Jolla, CA, U.S.A.). All other chemicals were purchased from Nakarai Chemicals Ltd (Kyoto, Japan).

Results

Following the addition of 1 μM dexamethasone, the β-adrenergic adenylate cyclase response of FRSK keratinocytes increased by 1·5-fold (Fig. 1). The prostaglandin E–adenylate cyclase response was similarly increased by treatment with dexamethasone. These stimulatory effects were usually detected after 6 h of incubation and remained for at least 48 h (Fig. 2a). Usually the difference was most marked after 12–24 h incubation. The adenosine–adenylate cyclase response was not affected by the glucocorticoid treatment (Fig. 1). In contrast to pig epidermis,[24,25] histamine had only a small effect on FRSK cells[14] which was not altered by the addition of dexamethasone (Fig. 1). The augmentation of the β-adrenergic response by dexamethasone was concentration-dependent and a maximal effect was observed at 0·5–1 μM (Fig. 2b), which was the same for the augmentation of the prostaglandin E response (data not shown).

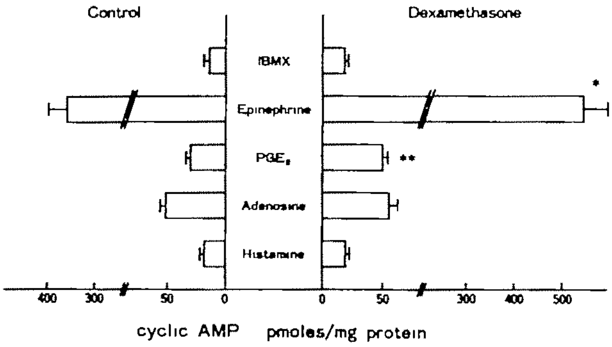

Figure 1. Effect of dexamethasone on epinephrine-, prostaglandin E$_2$-, adenosine-, and histamine-induced cyclic AMP accumulations of FRSK cells.

FRSK cells were treated with 1 μM dexamethasone for 24 h. Then the cells were treated with various adenylate cyclase stimulators for 5 min. The concentrations of chemicals were epinephrine, 50 μM; prostaglandin E$_2$, 30 μM; adenosine, 2 mM; and histamine 1 mM, respectively. Cyclic AMP phosphodiesterase inhibitors were added to the incubation media (1 mM IBMX for epinephrine, prostaglandin E$_2$, and histamine activation; 250 μM papaverine for adenosine activation). The effect of papaverine alone was similar to that of IBMX alone (not shown). All $n=4$ from a typical experimental series (Bars: SE). * $P<0{\cdot}05$ compared with control; ** $P<0{\cdot}01$ compared with control.

The effects of various steroids are shown in Table 1. Although dexamethasone increased the β-adrenergic adenylate cyclase response, neither testosterone, β-oestradiol, nor progesterone had any effect on the β-adrenergic response of FRSK cells.

The effect of dexamethasone on the β-adrenergic response of FRSK cells was completely inhibited by the RNA synthesis inhibitor, actinomycin D (Fig. 3a) and by the protein synthesis inhibitor, cycloheximide (Fig. 3b).

The β-adrenergic receptor of keratinocytes is the β_2-type.[13,14] A rise in the steady state β_2-adrenergic receptor mRNA levels was observed within 3 h of exposure of the FRSK cells to glucocorticoid (Fig. 4). The increased level of β_2-adrenergic receptor mRNA remained for up to 12 h incubation but had returned to the steady-state level after 24 h incubation. Thus, the increased levels of β_2-adrenergic receptor mRNA preceded the increased β-adrenergic response (Fig. 2a). Actin mRNA levels were not significantly altered during the 24-h treatment with dexamethasone (Fig. 4). Testosterone, β-oestradiol and progesterone had no effect on the β-adrenergic receptor mRNA levels (data not shown).

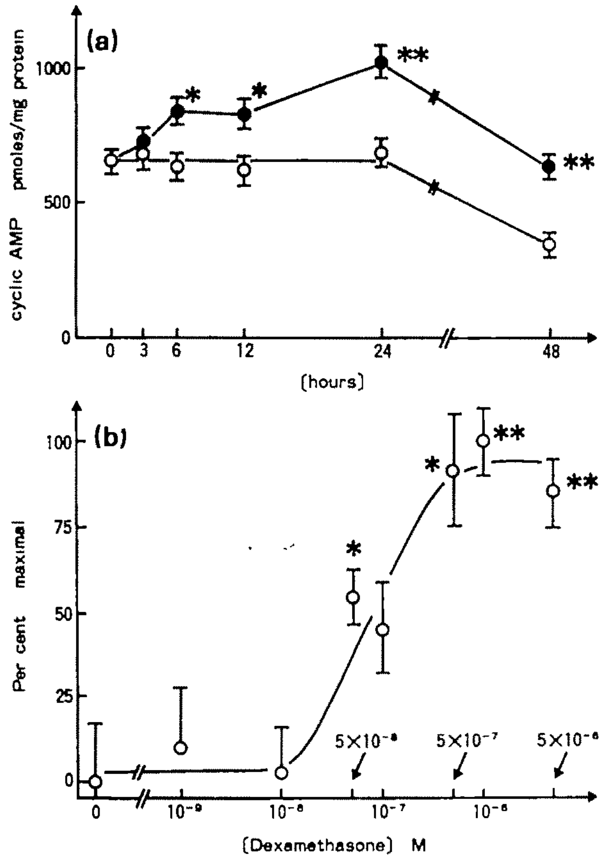

Figure 2. Effect of dexamethasone on the response to epinephrine by FRSK cells. (a) FRSK cells were incubated with 1 μM dexamethasone for up to 48 h. At the indicated time, cells were inccubated with 50 μM epinephrine in the presence of 1 mM IBMX for 5 min. o—o, control, ●—●, dexamethasone treated. (b) FRSK cells were incubated with various concentrations of dexamethasone for 24 h. Following the incubation, cells were further incubated with 50 μM epinephrine in the presence of 1 mM IBMX for 5 min. Data are presented as the per cent of stimulation achieved in response to 1 μM dexamethasone. Cyclic AMP accumulations of control cells and dexamethasone (1 μM)-treated cells were 700 pmoles/mg protein, and 1020 pmoles/mg protein, respectively. All $n=4$ from a typical experimental series (Bars; SE). * $P<0{\cdot}05$ compared with control; ** $P<0{\cdot}01$ compared with control.

Following treatment with the glucocorticoid, the levels of β-adrenergic receptors of FRSK cells were increased, and were visible using indirect immunofluorescence techniques (Fig. 5). This increase was consistently observed after treatment with dexamethasone. Again no increase in levels of the β-adrenergic receptor was observed following the incubation of FRSK cells with testosterone, β-oestradiol, or progesterone (data not shown).

Glucocorticoids may regulate G-proteins resulting in the altered function of adenylate cyclase.[26] Keratinocytes are known to express Gs as well as Gi-2 and Gi-3,

Table 1. Effects of various steroids on FRSK cells

	Cyclic AMP pmoles/mg protein			
	None	Epinephrine	Cholera toxin	Forskolin
Control	15±2	831±46	920±86	429±61
Dexamethasone	17±3	1312±102*	769±59	501±48
Testosterone	19±3	805±69	ND	ND
β-oestradiol	15±2	816±52	ND	ND
Progesterone	11±3	760±64	ND	ND

FRSK cells were incubated with various steroids (1 μM) or vehicle (control) for 24 h. Cells were then treated with 50 μM epinephrine for 5 min, 100 μg/ml cholera toxin for 2 h, and 100 μM forskolin for 1 h, respectively. 1 mM IBMX was added to the incubation medium for cyclic AMP accumulation. Data are the means±SE of a typical experimental series ($n=4$).
* $P<0{\cdot}01$ compared with control.
ND, not determined.

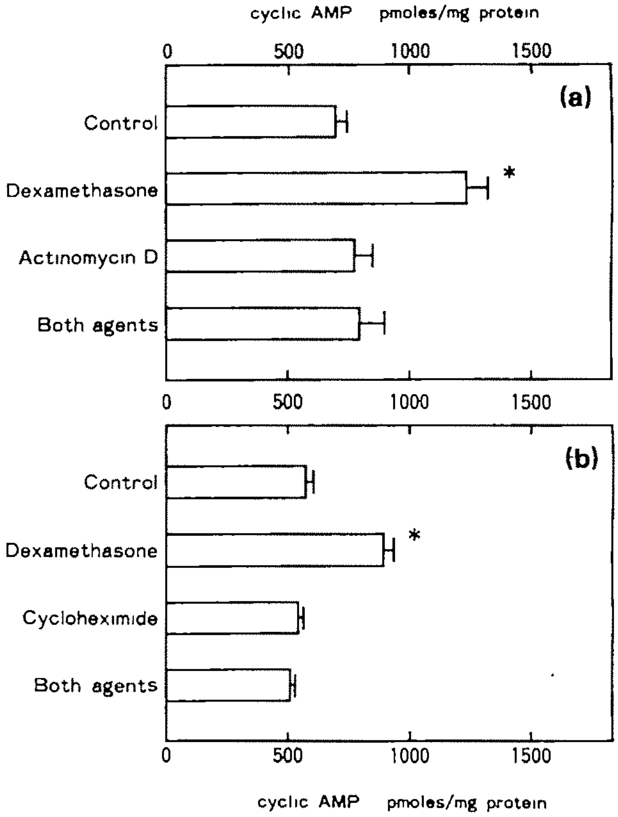

Figure 3. Effects of actinomycin D and cycloheximide on dexamethasone-induced β-adrenergic augmentation. (a) FRSK cells were incubated with 1 μM dexamethasone, and/or 5 μg/ml actinomycin D for 24 h. Cells were then treated with 50 μM epinephrine in the presence of 1 mM IBMX. (b) FRSK cells were incubated with 1 μM dexamethasone, and/or 0·5 μM cycloheximide for 24 h. Cells were then treated with 50 μM epinephrine in the presence of 1 mM IBMX. All $n=4$ from a typical experimental series (Bars; SE) * $P<0{\cdot}01$ compared with control.

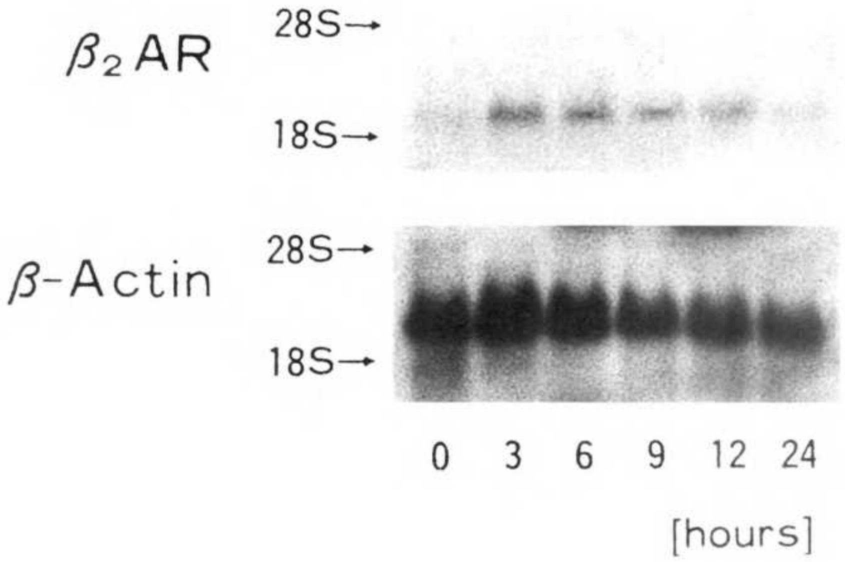

Figure 4. Effect of treatment with dexamethasone on β_2-adrenergic receptor and actin mRNA levels in FRSK cells. FRSK cells were treated with 1 μM dexamethasone for the indicated time, and RNA was prepared. From each time point 20 μg poly(A)$^+$ RNA were fractionated through a 1% agarose gel and blotted. Blots were probed with (a) [^{32}P]-labelled β_2-adrenergic receptor cDNA or (b) actin cDNA. There is a marked difference in the β_2-adrenergic receptor mRNA blot between 0 h and 3,6,9, 12 h. The β_2-adrenergic receptor mRNA returned to basal level by 24 h.

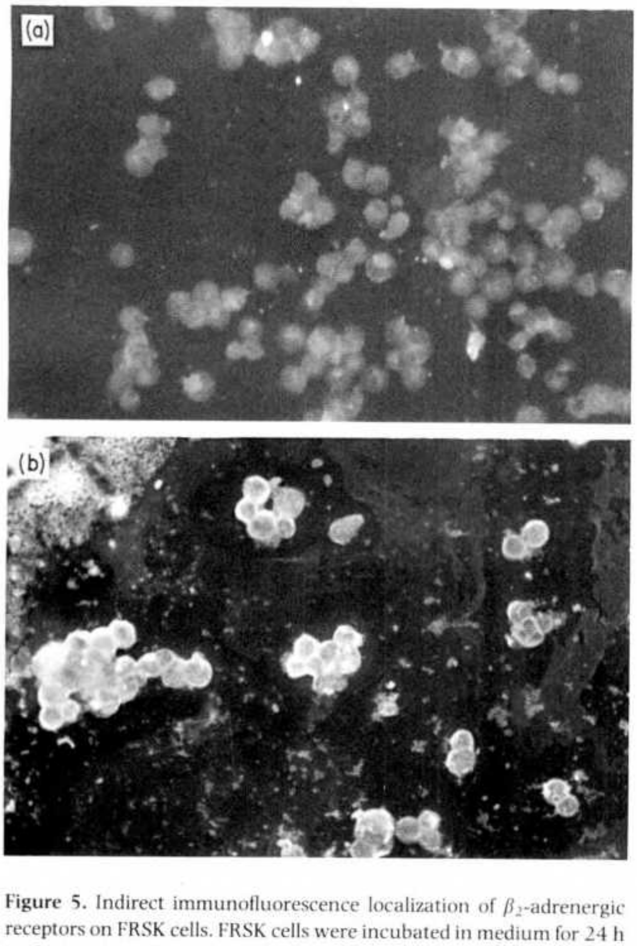

Figure 5. Indirect immunofluorescence localization of β_2-adrenergic receptors on FRSK cells. FRSK cells were incubated in medium for 24 h in the (a) absence or (b) presence of 1 μM dexamethasone. (× 200).

but not Gi-1.[14,17] Neither Gs-α, nor Gi-2α, Gi-3α mRNA levels were changed by the dexamethasone treatment (data not shown). Consistent with this finding, cholera toxin- and forskolin-induced cyclic AMP accumulations were not changed by the dexamethasone treatment (Table 1).

Discussion

Our results indicate that glucocorticoids augment the β-adrenergic adenylate cyclase response of FRSK cells. The effect was specific to the glucocorticoid; testosterone, β-oestradiol and progesterone had no effect. The prostaglandin E–adenylate cyclase response was also increased, but neither the adenosine–adenylate cyclase response nor the cholera toxin- or forskolin-induced cyclic AMP accumulations were changed by treatment with the glucocorticoid. The effect of the glucocorticoid on the β-adrenergic response was inhibited by actinomycin D and cycloheximide, which are inhibitors of protein synthesis at the transcription and translation step, respectively. These results are consistent with previous studies using normal pig epidermis.[1,2,25] Thus, FRSK cells were shown to have the β-adrenergic adenylate cyclase system regulated by glucocorticoids through the protein synthesis mechanism as in the normal epidermis.

In contrast to normal epidermis, FRSK cells express relatively high steady-state levels of β_2-adrenergic receptor mRNA.[14] Our results indicate that the β-adrenergic augmentation effect was preceded by an increase in the levels of β_2-adrenergic receptor mRNA. Although the increased receptor mRNA could be due either to increased transcription or to increased stability of the mRNA, it is known that for most steroid-responsive genes, expression is largely controlled by changes in the rate of transcription.[7–9] The increased level of β-adrenergic receptor was visualized using a monoclonal antibody specific for the β_2-adrenergic receptor.[23] In contrast, no evidence for regulation by G-proteins was detected in the present study, which is consistent with our previous results using normal pig epidermis.[17]

Thus, it is likely that glucocorticoids augment the β-adrenergic adenylate cyclase response through the enhanced expression of the β_2-adrenergic receptor in FRSK cells. To our knowledge this is the first report regarding the glucocorticoid-induced regulation of the β_2-adrenergic receptor in keratinocytes. Although a similar mechanism might be applicable to the prostaglandin E–adenylate cyclase response, without a probe for the prostaglandin E receptor, this cannot yet be determined.

It has been reported that the β-adrenergic adenylate cyclase response is decreased in various pathological conditions affecting keratinocytes. The involved epidermis in psoriasis and the hyperproliferative epidermis induced by the phorbol ester TPA are examples[27,28] in which glucocorticoids reveal various antagonistic effects.[6,29,30] The β-adrenergic augmentation effect, which appears to depend on the transcriptional control of the β_2-adrenergic receptor, may have significant effects on keratinocytes through an alteration of their adenylate cyclase–cyclic AMP system.

Acknowledgments

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References

1 Iizuka H, Ohkawara A. Effects of glucocorticoids on the beta-adrenergic adenylate cyclase system of pig skin. *J Invest Dermatol* 1983; 80: 524–8.

2 Ohkawara A, Iizuka H. Glucocorticoid-induced alteration of beta-adrenergic adenylate cyclase response of epidermis *Arch Dermatol Res* 1985; 277: 88–92.

3 Iizuka H, Kishiyama N, Ohkawara A. Modulation of pig epidermal adenylate cyclase responses by protein synthesis inhibitors: its relation to glucocorticoid and colchicine effect. *Arch Dermatol Res* 1985; 277: 466–72.

4 Huang K-S, McGray P, Mattaliano RJ *et al.* Purification and characterization of proteolytic fragments of lipocortin I that inhibit phospholipase A2. *J Biol Chem* 1987, 262: 7639–45.

5 Cartwright PH, Ilderton E, Sowden JM, Yardley HJ. Inhibition of normal and psoriatic epidermal phospholipase A2 by picomolar concentrations of recombinant human lipocortin I. *Br J Dermatol* 1989; 121: 155–60.

6 Iizuka H, Kajita S, Mizumoto T, Kawaguchi H. Glucocorticoid-induced modulation of the beta-adrenergic adenylate cyclase response of epidermis: its relation to epidermal phospholipase A2 activity. *J Invest Dermatol* 1986; 87: 577–81.

7 Malbon CC, Hadcock JR. Evidence that glucocorticoid response elements in the 5′-noncoding region of the hamster β_2-adrenergic receptor gene are obligate for glucocorticoid regulation of receptor mRNA levels. *Biochem Biophys Res Commun* 1988; 154: 676–81.

8 Collins S, Caron MG, Lefkowitz RJ. β_2-Adrenergic receptors in hamster smooth muscle cells are transcriptionally regulated by glucocorticoids. *J Biol Chem* 1988; 263: 9067–70.

9 Hadcock JR, Malbon CC. Regulation of β-adrenergic receptors by 'permissive' hormones: glucocorticoids increase steady-state levels of receptor mRNA. *Proc Natl Acad Sci USA* 1988; 85: 8415–19.

10 Chung F-Z, Lentes K-U, Gocayne J *et al.* Cloning and sequence analysis of the human brain β-adrenergic receptor. *FEBS Lett* 1987; 211: 200–6.

11 Kobilka BK, Frielle T, Dohlman HG *et al.* Delineation of the intronless nature of the genes for the human and hamster β_2-adrenergic receptor and their putative promoter regions. *J Biol Chem* 1987; 262: 7321–7.

12 Emorine LJ, Marullo S, Delavier-Klutchko C *et al.* Structure of the gene for human β_2-adrenergic receptor: expression and promoter characterization. *Proc Natl Acad Sci USA* 1987; 84: 6995–9.

13 Duell EA. Identification of a beta$_2$-adrenergic receptor in mammalian epidermis. *Biochem Pharmacol* 1980; 29: 97–101.

14 Takahashi H, Tamura T, Tsutsui M, Iizuka H. Adenylate cyclase system in FRSK cells and SV40-transformed human keratinocytes. *J Dermatol (Tokyo)* 1990; 17: 457–64.

15 Indo K, Miyaji H. Qualitative changes in the biologic characteristics of cultured fetal rat keratinizing epidermal cells during the process of malignant transformation after benzo[a]pyrene treatment. *J Natl Cancer Inst* 1979; 63: 1017–27.

16 Tsutsui M, Iizuka H, Ohkawara A *et al.* Adenylate cyclase system of human trichilemmoma cell line. *Arch Dermatol Res* 1987; 279: 530–5.

17 Matsuo S, Iizuka H. Cholera toxin-, and forskolin-induced cyclic AMP accumulations of pig skin (epidermis): modulation by chemicals, which reveal the beta-adrenergic augmentation effect. *J Dermatol Sci* 1990; 1: 7–14.

18 Maniatis T, Fritsch EF, Sambrook T. *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, 1989.

19 Thomas PS. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc Natl Acad Sci USA* 1980; 77: 5201–4.

20 Kobilka B, Dixon R, Frielle T *et al.* cDNA for human β_2-adrenergic receptor. *Proc Natl Acad Sci USA* 1987; 84: 46–50.

21 Kozasa T, Itoh H, Tsukamoto T, Kaziro Y. Isolation and characterization of the human Gs-α gene. *Proc Natl Acad Sci USA* 1988; 85: 2081–5.

22 Itoh H, Toyama R, Kozasa T *et al.* Presence of three distinct molecular species of Gi protein α subunit. *J Biol Chem* 1988, 263: 6656–64.

23 Kaveri S, Cervantes-Olivier P, Delavier-Klutchko C, Strosberg AD. Monoclonal antibodies directed against the human A431 β_2-adrenergic receptor recognize two major polypeptide chains. *Eur J Biochem* 1987; **167:** 449–56.

24 Iizuka H, Adachi K, Halprin KM, Levine V. Histamine (H_2) receptor adenylate cyclase system in pig skin (epidermis). *Biochim Biophys Acta* 1976; **437:** 150–7.

25 Koizumi H, Yasui C, Shimizu T, Ohkawara A. Stimulation of prostaglandin E-adenylate cyclase response in pig epidermis by hydrocortisone. *Arch Dermatol Res* 1989; **281:** 215–17.

26 Garcia-Sainz JA, Huerta-Bahena ME, Malbon CC. Hepatocyte β-adrenergic responsiveness and guanine nucleotide-binding regulatory proteins. *Am J Physiol* 1989; **256:** C384–9.

27 Iizuka H, Adachi K, Halprin KM, Levine V. Cyclic AMP accumulation in psoriatic skin: differential responses to histamine, AMP, and epinephrine by the uninvolved and involved epidermis. *J Invest Dermatol* 1978; **70:** 250–3.

28 Iizuka H, Sakai H, Tamura T. Effects of the tumor promoter, phorbol 12-myristate, 13-acetate, on the epidermal adenylate cyclase system: evidence for adenylate cyclase-regulation by protein kinase C. *J Invest Dermatol* 1989; **93:** 387–91.

29 Fredriksson T, Lassus A, Bleeker J Treatment of psoriasis and atopic dermatitis with halcinonide cream applied once and three times daily. *Br J Dermatol* 1980; **102:** 575–7.

30 Davidson KA, Slaga TJ. Effects of phorbol ester tumor promoters and hyperplasiogenic agents on cytoplasmic glucocorticoid receptors in epidermis. *J Invest Dermatol* 1982; **79:** 378–82.

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Histiocytes in Sweet's syndrome

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Summary

The histological features of biopsies from 18 previously unreported cases of Sweet's syndrome are reported. The dermal infiltrate in the majority of the cases contained numerous histiocytes that at first sight appeared to mimic neutrophils. The immunophenotype of these histiocytes was consistent with monocytes that have freshly infiltrated into the lesions. Only two of the cases in this series, associated with leukaemia, displayed the histological features of Sweet's syndrome with a predominant neutrophilic infiltration.

We suggest that the initiating mechanisms in Sweet's syndrome are that monocyte/histiocyte-derived cytokines such as the interleukins IL-1 and IL-8, secreted either by infiltrating histiocytes in the non-leukaemia-associated cases of Sweet's syndrome or by tumoural myelomonocytic cells in those associated with leukaemia, are responsible for the systemic manifestations and the infiltration with neutrophils in the skin lesions.

Acute febrile neutrophilic dermatosis or Sweet's syndrome was first described in 1964 by Sweet[1] and since then there have been many further reports. The syndrome is well-recognized and the diagnostic criteria have been defined.[2] The disease is mainly characterized by tender plaques or nodules associated with fever and there is an infiltrate of neutrophils in the dermis in the lesions. The latter finding has been stressed as being the salient histological feature.[1] A recent histopathological study carried out on a large series of cases has indicated the diversity of the dermal infiltrate and recognized lymphocytic, neutrophilic and histiocytic stages.[3] The pathogenesis of the disease is as yet not known, but based on the number of conditions associated with Sweet's syndrome, both malignant and inflammatory,[4] it is thought to represent a hypersensitivity reaction to a variety of antigens. Several authors suggest that it is due to a reaction to immune complexes, but these have not been demonstrated either in the circulation or in the skin lesions.[5] Also, there is evidence that complement does not play a role in neutrophil chemotaxis in cases of Sweet's syndrome.[6] Recently it has been suggested that IL-1 is the inflammatory mediator in leukaemia-associated Sweet's syndrome.[7]

This present study has analysed the histopathological findings in 18 cases of Sweet's syndrome with particular emphasis on the types of infiltrating cells in the lesions.

Methods

Eighteen patients with a diagnosis of Sweet's syndrome from 1979 to 1989 were studied and this study was based on their clinical and histological data (Table 1).

In 10 of the cases, half of the skin biopsy was snap frozen in liquid nitrogen and isopentane and was stored at −70°C until used. Portions of these and biopsies from eight other cases were fixed and processed for routine histology. According to the antigen to be detected, either immunoperoxidase or immunofluorescence techniques were used on either the fixed or frozen tissue sections. Immunoperoxidase techniques included a three-step peroxidase–antiperoxidase method and a three-step avidin–biotin complex method as have been previously described.[8] FITC-labelled antibodies were used for direct immunofluorescence. The monoclonal and polyclonal antibodies used in this study are listed in Table 2.

Results

Histopathology

The epidermis was intact in most of the biopsies although there was occasional spongiosis. Exocytosis of inflammatory cells into the epidermis was not a feature.

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Table 1. Clinical and histological data

Sex	Age	Associated conditions	Localization of the lesions	Signs and symptoms	Treatment	A	B	C
F	56	Arthralgia	Arms, trunk, legs	Fever	Prednisolone 25 mg/day	R	NR	H/N
F	34	Bronchitis	Arms, trunk, legs	Fever 38°, neutrophilia, myalgia	Prednisolone 30 mg/day	SR	2	H/N
F	42	Pharyngitis	Face, arms, legs	Fever 39·6°, neutrophilia, myalgia	Prednisone 30 mg/day	R	5	H
M	63	NR	Face, arms, trunk, legs	Spiking fever, neutrophilia	Prednisolone 30 mg/day	R	2	H
M	65	Pharyngitis	Face, arms, trunk	Fever 38°, neutrophilia, conjunctivitis	No	SR	7	H
M	75	AML(M4)	Neck, arms, trunk	Fever	Cefazoline	R*	14	N
M	64	Gastro-enteritis	Arms, legs	Fever 38·5°, neutrophilia, erythema nodosum	Prednisolone 25 mg/day	R	2	H
F	71	Cystitis	Arms, legs	Fever 38·5°	Prednisolone 30 mg/day	R	5	H/N
M	57	NR	Face, arms, legs	Fever 39°, myalgia, iritis	Prednisolone 25 mg/day	R	10	H/N
F	79	Sjögrens disease	Face, arms, legs	Fever	Prednisolone 25 mg/day	NR	12	H
F	43	NR	Arms, legs	Fever 38°, neutrophilia	Prednisolone 25 mg/day	R	3	H
F	31	NR	Face, arms, legs	Fever, neutrophilia, arthralgia	Prednisone 30 mg/day	R	2	H
F	45	Tonsilitis	Face, arms, legs	Fever 39°, neutrophilia, arthralgia	Prednisone 30 mg/day	R	3	H/N
M	73	AML(M4)	Arms, trunk, legs	Fever	NR	NR	NR	N
F	58	NR	Arms, trunk, legs	Fever 39°, neutrophilia	Prednisolone 30 mg/day	R	7	H
F	49	Pharyngitis	Arms, shoulders, knees	Fever 38·7°, myalgia	No	SR	2	H
M	60	Pharyngitis, Carcinoma of the bladder	Neck, shoulders, arms, trunk	Fever 38·5°, neutrophilia, myalgia, conjunctivitis	No	SR	7	H
F	56	Pharyngitis	Arms, shoulders, pubis, knees	Fever 38°C, neutrophilia	Prednisolone 25 mg/day	R	4	H

A, outcome; B, age of the lesion (days), C, composition of the inflammatory infiltrate; H, predominantly histiocytic; H/N, mixed histiocytic and neutrophilic; N, predominantly neutrophilic; R, remission; SR, spontaneous regression; NR, not recorded; * remission after 6 weeks.

Table 2. Antibodies used in this study

Antibody	Specificity	Working dilution	Source
EBM11(m)	CD68, monocytes/histiocytes	1:10	Dakopatts
KP1(m)	CD68, monocytes/histiocytes	1:10	Dakopatts
RFD7(m)	Histiocytes	1:20	Dr Poulter (London)
RFD9(m)	Histiocytes	1:50	Dr Poulter (London)
Leu M3(m)	CD14, monocytes, histiocytes	1:25	Becton-Dickinson
Anti-AACT(p)	Monocytes, histiocytes	1:500	Dakopatts
Anti-factor XIII(p)	Monocytes, histiocytes	1:100	Behring-Werke
Anti-MRP8(p)	Monocytes, histiocytes	1:1000	Dr Brüggen (Basel)
Anti-MRP14(p)	Monocytes, histiocytes	1:4000	Dr Brüggen (Basel)
OKT6(m)	CD1	1:50	Ortho Diagnostics
NP57(m)	Neutrophils	1·10	Dr D.Y Mason (Oxford)
TAL1B5(m)	HLA-DR	1:05	Dr W.F.Bodmer (London)
MT1(m)	CD43, T lymphocytes	1:10	Clonab
L26(m)	B lymphocytes	1:250	Dakopatts
Anti-C3(m)	C3	1:80	Behring Werke
Anti-C1q(m)	C1q	1:40	Dakopatts
Anti-IgA(m)	IgA	1:40	Dakopatts
Anti-IgM(m)	IgM	1:40	Dakopatts
Anti-IgG(m)	IgG	1:80	Dakopatts

m, monoclonal; p, polyclonal.

All the biopsies showed slight to moderate oedema of the papillary dermis with aggregates or clusters of inflammatory cells in the upper and mid-dermis. Occasionally there was a diffuse dermal infiltrate. In two of the biopsies, the infiltrate was present in the deep part of the dermis. In none of the biopsies was there any infiltration of the subcutaneous tissue. The infiltrate varied in its composition, and in two of the cases was predominantly neutrophilic (Figs 1a and b). In 11 cases the infiltrate consisted mainly of histiocytes (Fig. 1c). In the remaining cases there were equal numbers of neutrophils and histiocytes. Lymphocytes were present in all the skin biopsies but were clearly outnumbered by other inflammatory cells. There was no relationship between the duration of the lesions and the composition of the cellular infiltrate. The histiocytes had a typical morphology, being small and round with an elongated lobate nucleus and with a limited amount of cytoplasm (Fig. 2). These histiocytes appeared to mimic neutrophils, but lacked a multilobular nucleus. In those lesions with a predominantly histiocytic infiltrate, clusters of histiocytes were found which showed in their centre a cleft or

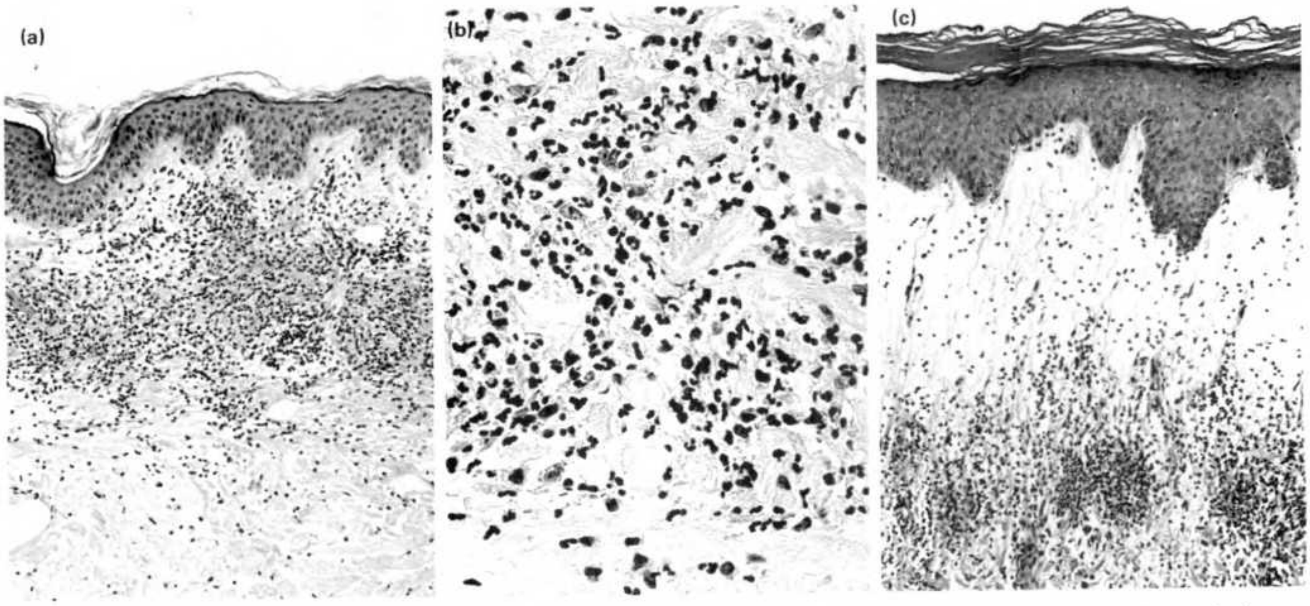

Figure 1. (a) Skin biopsy showing diffuse infiltrate in the upper dermis, almost entirely composed of neutrophils (b). This patient had acute myelomonocytic leukaemia (M4). (c) Skin biopsy showing superficial oedema and a nodular infiltrate in the dermis (paraffin sections, × 160, × 640, × 160, respectively).

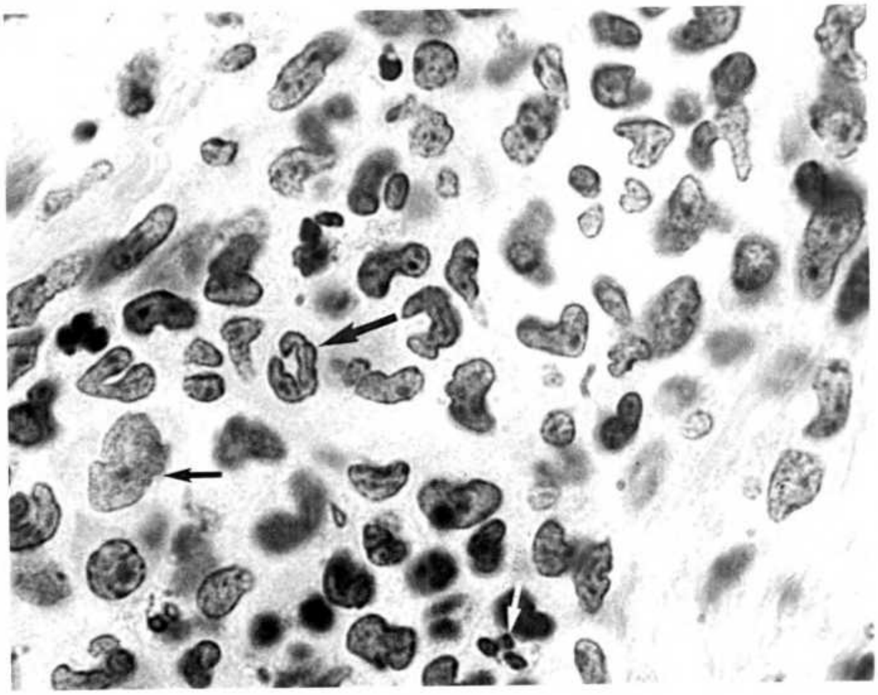

Figure 2. Small histiocytes observed in the majority of the lesions in Sweet's syndrome have elongated, twisted nuclei (large black arrow). Some larger histiocytes with an oval nucleus (small black arrow) and some neutrophils (white arrow) can also be seen (paraffin section, × 1600).

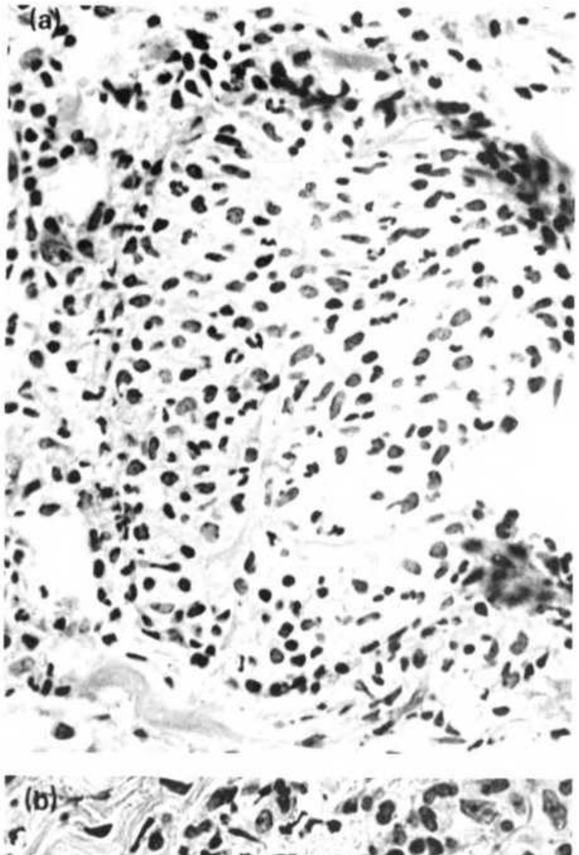

Figure 3. (a) Miescher radial-like granuloma in Sweet's syndrome. Small histiocytes surround a central cleft in a more or less radial configuration. Few neutrophils are interspersed. (b) Cluster of cells. The centre is composed of neutrophils, while the periphery is composed of small histiocytes (paraffin sections, × 400).

vessel (Fig. 3a). In the cases with an admixture of neutrophils and histiocytes, the histiocytes were virtually always at the periphery, whereas the neutrophils occupied the centre of the clusters (Fig. 3b). Occasionally a vessel could be seen at the centre of these aggregates. There was no evidence of a vasculitis in any of the cases and leucocytoclasia was observed to a limited degree in those lesions with a predominantly neutrophilic infiltrate.

Immunohistology

Histiocytes and neutrophils were distinguished on the basis of elastase positivity of neutrophils (Fig. 4a). The histiocytes were stained for CD68, CD14, Factor XIIIa, AACT, MRP8 and MRP14 (Figs 4b and c) but not for CD1, RFD7 or RFD9. The HLA-DR expression varied between the cases, but was generally weak. The lymphocytes were mainly T cells as shown by their positivity with MT1 (CD43). Only a few L26 positive B cells were present.

Immunofluorescence studies did not reveal any significant deposition of complement and immunoglobulins in the vessels or at the dermo-epidermal junction.

Discussion

The main histological feature of Sweet's syndrome is a dermal infiltrate of neutrophils in lesions without evidence of a vasculitis.[1,9,10] In a recent study[3] lymphocytic, neutrophilic and histiocytic stages of the lesions in Sweet's syndrome have been described. These were based on the clinical appearances of the lesions at the time of biopsy and it was suggested that the lymphocytic stage might be first and followed by a neutrophilic and then a histiocytic stage. The findings in our study do not support this, because a predominantly histiocytic infiltrate was noted in most of the cases. We wish to stress that although at first glance the cells may appear to be neutrophils, they did not stain for neutrophil elastase but, as expected, expressed pan-histiocyte markers CD68, CD14, AACT and Factor XIIIa, as well as MRP8 and MRP14 that are two recently described monocyte/histiocyte markers.[11] CD1 and antigens recognized by RFD7 and RFD9[12] were not expressed. These findings suggest that the histiocytes in the lesions in Sweet's syndrome represent monocytes that have freshly infiltrated.

The histiocytic clusters seen in Sweet's syndrome resembled those of Miescher's radial granulomas which are characteristically seen in erythema nodosum.[13] The

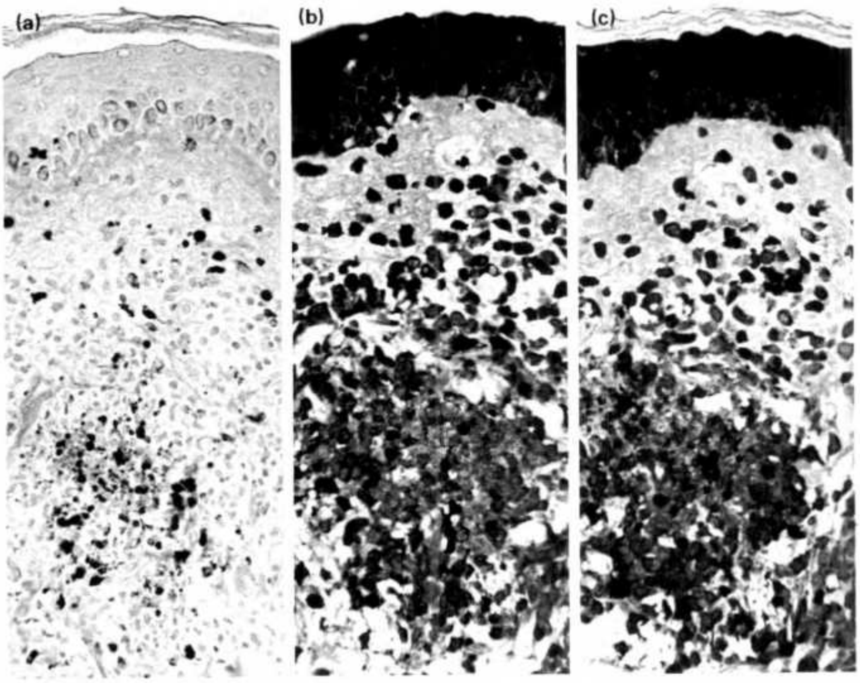

Figure 4. Staining for (a) neutrophil elastase, (b) MRP8 and (c) MRP14 in serial sections of a skin biopsy of mixed neutrophilic and histiocytic Sweet's syndrome. Neutrophils in the centre of the infiltrate are stained for neutrophil elastase and MRP8 and MRP14. Histiocytes are stained for MRP8 and MRP14, but not for neutrophil elastase. The epidermis stains for MRP8 and MRP14 (paraffin sections, ×400).

immunophenotype of the histiocytes in both types of lesions is similar.[14] The pathogenesis of erythema nodosum remains unknown, but an Arthus type reaction with the local formation of immune complexes and subsequent complement activation with chemotaxis of inflammatory cells probably plays a role in the causation of these lesions.[15] A similar phenomenon may also occur in Sweet's syndrome. Although deposition of immune complexes was not demonstrated in our series of patients, nor in previous studies,[5] this may be due to their rapid clearance by infiltrating cells. Chemoattractants other than complement might 'trigger' the lesions in Sweet's syndrome, as has been described in a patient with profound deficiency of C3.[6]

In the present study an infiltrate that consisted only of neutrophils was found in only two cases that both had an associated leukaemia. Production of IL-1 by neoplastic cells might be responsible for the infiltrate of neutrophils.[7] IL-1 has been reported to be produced *in vitro* by the cells in myelomonocytic and monocytic leukaemia[16–18] and these are the types of acute non-lymphoblastic leukaemia that are most frequently associated with Sweet's syndrome.[19] The neutrophil component of the infiltrate in cases not associated with leukaemia may similarly be ascribed to monocyte-derived cytokines such as IL-1 and the recently characterized IL-8[20] and be secreted by the histiocytes.

In conclusion, Sweet's syndrome is characterized by a predominantly histiocytic infiltrate that shows the characteristics of monocytes that have freshly infiltrated into the lesion and which could be misinterpreted as being neutrophils in routine stained sections. In our series of patients the classical infiltrate with neutrophils was observed only in those cases that were associated with leukaemia.

Acknowledgments

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References

1 Sweet RD. An acute febrile neutrophilic dermatosis. *Br J Dermatol* 1964; **76:** 349–56.

2 Su WPD, Liu HNH. Diagnostic criteria for Sweet's syndrome. *Cutis* 1986; **37:** 167–74.

3 Jordaan HF. Acute febrile neutrophilic dermatosis: a histopathological study of 37 patients and a review of the literature. *Am J Dermatopathol* 1989; **11:** 99–111.

4 Cohen PR, Talpaz M, Kurzrock R. Malignancy-associated Sweet's syndrome: review of the world literature. *J Clin Oncol* 1988; **6:** 1887–97.

5 Going JJ, Going SM, Myskow MW, Beveridge GW. Sweet's syndrome: histological and immunohistochemical study of 15 cases. *J Clin Pathol* 1987; **40:** 175–9.

6 Weiss RM, Schulz EJ. Complement deficiency in Sweet's syndrome. *Br J Dermatol* 1989; **121**: 413–5

7 Going JJ. Is the pathogenesis of Sweet's syndrome mediated by interleukin-1? *Br J Dermatol* 1987; **116**: 282–3

8 Hsu SM, Raine L, Fanger H. The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase technique. a comparison between ABC and unlabelled antibody (PAP) procedures. *J Histochem Cytochem* 1981, **29**: 577–80.

9 Lever W, Schaumburg-Lever U. *Histopathology of the Skin*, 6th edn. Philadelphia: JB Lippincot Company, 1983.

10 Ackerman AB *Histologic Diagnosis of Inflammatory Skin Diseases*. Philadelphia: Lea & Febiger, 1978.

11 Odink K, Cerletti N, Bruggen J *et al.* Two calcium-binding proteins in infiltrate macrophages of rheumatoid arthritis. *Nature* 1987; **330**: 80–2

12 Munro CS, Campbell DA, Collings LA, Poulter LW. Monoclonal antibodies distinguish macrophages and epitheloid cells in sarcoidosis and leprosy. *Clin Exp Immunol* 1987, **68**: 282–7.

13 Sanchez Yuz E, Sanz Vico MD, de Diego V. Miescher's radial granuloma: a characteristic marker of erythema nodosum. *Am J Dermatopathol* 1989; **11**: 434–42

14 Delabie J, De Wolf-Peeters C, Van den Oord JJ, Desmet V. Differential expression of the calcium binding proteins MRP8 and MRP14 in granulomatous conditions. *Clin Exp Immunol* 1990; **81**: 123–6.

15 Niemi KM, Forstrom L, Hannuksela M *et al.* Nodules on the legs. *Acta Derm Venereol* 1977; **57**: 145–54.

16 Furukawa Y, Ohta M, Miura Y, Saito M. Interleukin-1 producing ability of leukemia cells and its relationship to morphological diagnosis. *Br J Haematol* 1987; **65**: 11–5.

17 Griffin JD, Rambaldi A, Vellenga E *et al.* Secretion of interleukin-1 by acute myeloblastic leukemia cells *in vitro* induces endothelial cells to secrete colony stimulating factors. *Blood* 1987; **70**: 1218–21.

18 van der Schoot CE, Jansen P, Poorter M *et al.* Interleukin-6 and interleukin-1 production in acute leukemia with monocytoid differentiation *Blood* 1989; **74**: 2081–7.

19 Cooper PH, Innes DJ, Greer KE Acute febrile neutrophilic dermatosis (Sweet's syndrome) and myeloproliferative disorders. *Cancer* 1983; **51**: 1518–26.

20 Rampart M, Van Damme J, Zonnekeyn L, Herman AG Granulocyte chemotactic protein/interleukin-8 induces plasma leakage and neutrophil accumulation in rabbit skin. *Am J Pathol* 1989, **135**: 21–5.

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The effect of topical nitroglycerin on transcutaneous oxygen

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Summary The use of topical nitroglycerin has been reported to aid the healing of chronic skin ulcers. In this study, the effect of 2% nitroglycerin ointment on transcutaneous oxygen pressure (TcPO$_2$) was investigated in skin adjacent to the area of ointment application. Topical nitroglycerin caused a two–fivefold decrease in TcPO$_2$ in skin next to or up to 9 cm away from the site of application of the ointment.

Nitroglycerin has long been used to treat angina pectoris. In spite of recent advances in cardiovascular medicine, it remains a drug of choice for coronary artery insufficiency. When injected in the coronary bed during angiography, nitroglycerin relieves spasms and dilates the large epicardial coronary arteries. However, smaller transmural vessels are less affected, a possible advantage in preventing 'steal' from high resistance areas of the ischaemic myocardium. In clinical practice the plasma concentrations achieved with oral and topical nitrates do not allow a direct effect on the coronary arteries, but rather cause dilation of the systemic venous and arterial bed. These changes decrease preload and, to a certain extent, afterload on the ischaemic myocardium.[1,2]

Nitroglycerin was first used in topical form in 1955, and became available as a 2% ointment in 1974. For almost two decades, reports have appeared suggesting that topical nitroglycerin may be useful in the healing of chronic skin ulcers. It is possible that the use of topical nitroglycerin for this purpose is more widespread than indicated by these few published studies. We therefore decided to study the effect of topical nitroglycerin on transcutaneous oxygen pressure (TcPO$_2$), a parameter which is used clinically in the evaluation and management of chronic skin ulceration.[3,4] Specifically, we were interested in knowing whether the application of nitroglycerin to one area of skin would cause a 'steal' of oxygen from adjacent sites.

Methods

A total of five healthy volunteers (four males, one female, age range 23–32 years) participated in the study. The subjects were studied in the supine position. Initially, we attempted to study forearm TcPO$_2$ but baseline values at this site were consistently too low for meaningful results. We then investigated TcPO$_2$ at the infraclavicular area. Four TcPO$_2$ monitors (Radiometer TCM204, Copenhagen, Denmark) were used to measure four skin sites simultaneously. The first adhesive sensor (A) was placed over the mid-sternum. The remaining three sensors (B, C, and D) were placed successively in the second intercostal space on the left side of the chest, at a distance of 1·0 cm from sensor A and from each other (Fig. 1). After the sensor rings were in place, two drops of a contact solution (Radiometer) were placed inside each ring. Electrodes from individual TcPO$_2$ monitors were then connected to each ring. TcPO$_2$ measurements were taken after 20 min, at a sensor temperature of 37°C. After baseline TcPO$_2$ readings were obtained, 2% nitroglycerin (Nitro-Bid ointment, Marion Corp, Kansas City, Mo, U.S.A.) was applied in or around the first, midsternal sensor (A). TcPO$_2$ measurements were then recorded at all four sites (A, B, C, D) after an additional 20 min. In some experiments, the ointment was applied liberally to the skin to simulate the clinical situation. In other experiments, 1 mm of ointment, corresponding to approximately 0·6 g of 2% nitroglycerin, was applied.

Results

Nitroglycerin applied around sensor A

Application of a liberal amount of nitroglycerin ointment around sensor A caused a dramatic decrease in TcPO$_2$ inside sensor A and all adjacent sites. Figure 2 shows the representative results of one of the experiments in one subject. Sensors A, B, C, and D recorded a

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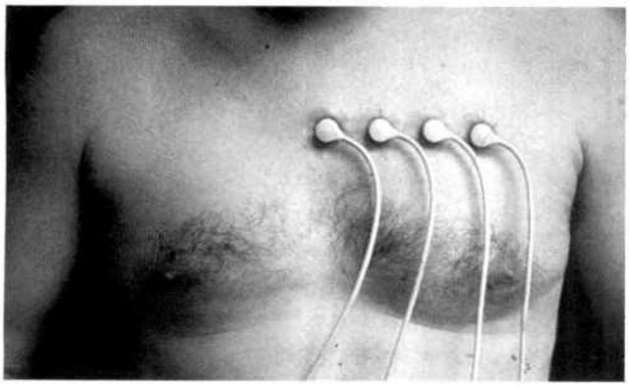

Figure 1. Four TcPO$_2$ sensors on left side of chest. The sensor near the mid-sternal area was labelled A, and adjacent sensors were labelled consecutively B to D.

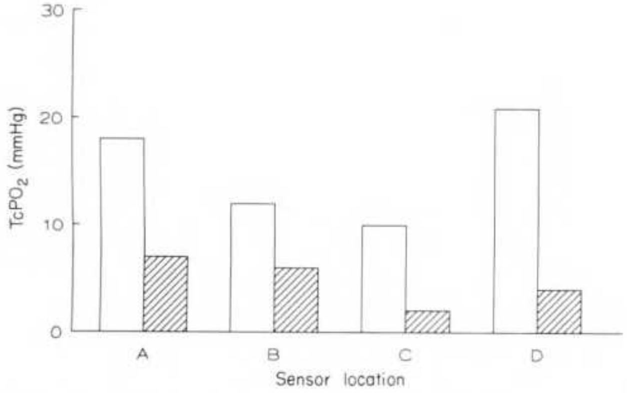

Figure 2. TcPO$_2$ measurements at 37°C before and after the application of a liberal amount of nitroglycerin ointment immediately around sensor A. □, baseline; ▨, after NTG.

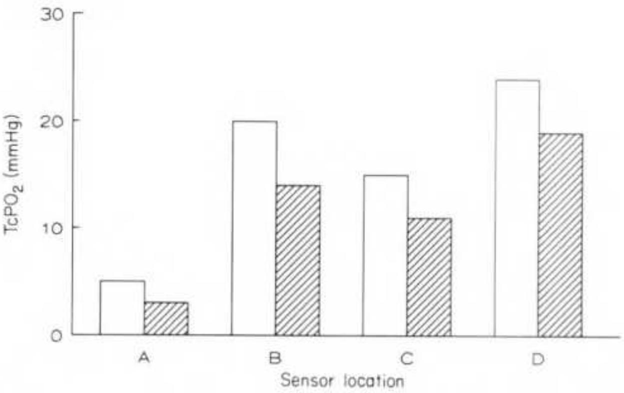

Figure 3. TcPO$_2$ measurements at 37°C before and after the application of 0·6 g of nitroglycerin ointment immediately around sensor A. □, baseline; ▨, after NTG.

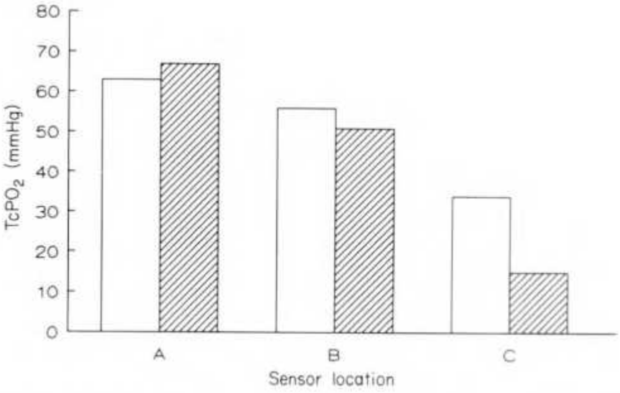

Figure 4. TcPO$_2$ measurements at 42°C before and after the application of 0·6 g of nitroglycerin ointment immediately around sensor A. □, baseline; ▨, after NTG.

260–500% reduction in TcPO$_2$. Baseline TcPO$_2$ measurements were variable at the different chest sites. We are not certain why adjacent skin areas should record different TcPO$_2$ values, but the influence of epidermal thickness,[5] vasculature, and subcutaneous tissue are likely to be important. Nevertheless, our comparisons were always made with respect to baseline values and within the same sensor location. When the amount of topical nitroglycerin ointment was reduced to 1 mm (0·6 g), the results were less dramatic, but a TcPO$_2$ decrease away from the application site was still observed (Fig. 3). For all patients tested, the TcPO$_2$ measurements obtained after the application of nitroglycerin and expressed as mean percentage of control (before nitroglycerin) ± SD were as follows: 59 ± 16 at sensor A, 49 ± 18 at B, 47 ± 22 at C, and 51 ± 24 at D. These decreases in TcPO$_2$ were statistically significant ($P<0{\cdot}01$) using Student's *t*-test. It should be noted that a sensor temperature greater than 37°C, which increases TcPO$_2$ readings to maximal levels, tends to minimize possible changes in TcPO$_2$ due to vessel tone. Nevertheless, after nitroglycerin application around sensor A and at a sensor temperature of 42°C, we observed a small but consistent decrease in TcPO$_2$ at distal sites (Fig. 4).

Nitroglycerin ointment applied inside sensor A

A study of the effects of the application of nitroglycerin inside the sensor, in actual contact with the electrode, may not be technically possible. We found that nitroglycerin increased TcPO$_2$ at sensor A by 300% of baseline (Fig. 5). However, a more than twofold increase was observed at sensor A after applying vasoline inside the sensor (data not shown). Sensors B, C, and D, reported

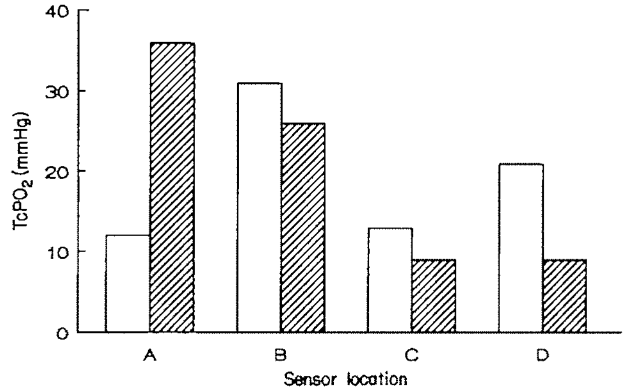

Figure 5. $TcPO_2$ measurements at 37°C before and after the application of 0·6 g of nitroglycerin ointment inside sensor A. □, baseline; ■, after NTG

decreased $TcPO_2$ values, as in previous experiments (Fig. 5).

In all these experiments, topical nitroglycerin did not produce any weal and flare, clinical vasodilation, or oedema.

Discussion

In spite of recent advances in wound care and effective topical and systemic antibiotics, the fundamental problems leading to chronic skin ulceration are not easily corrected. Ischaemia appears to cause or perpetuate most chronic ulcers, such as those associated with arterial insufficiency, pressure, and microvascular damage. Thus, topical agents that could selectively increase blood flow at the ulcer site are an attractive therapeutic modality. Topical nitroglycerin is an effective cutaneous vasodilator and there have been isolated reports suggesting that nitroglycerin ointment can help heal chronic ulcers. Wheeland *et al.*[6] reported that the use of topical nitroglycerin in three patients with chronic digital ulcerations from various causes led to healing of the ulcers in all three patients. Rinieri reported that the use of topical nitroglycerin in the wounds was followed by improvement in 10 patients with arterial or venous ulcers.[7] Topical use of nitroglycerin has been suggested in the treatment of Raynaud's disease and trophic ulcers. However, no controlled prospective studies have been done, although one prospective study did find that topical nitroglycerin improved the symptoms of intermittent claudication in patients with arterial insufficiency but without ulcers.[8]

In this study, we demonstrated the effect of topical nitroglycerin on oxygen delivery to the skin, as measured by transcutaneous oxygen ($TcPO_2$). $TcPO_2$ measurements have generally been recorded using a sensor temperature of either 37°C or 42–45°C. The latter temperatures, which are used more frequently, cause maximal vasodilation and, thus, maximal $TcPO_2$. A temperature of 37°C allows a more physiological vessel tone and was therefore used in this study. Initially, we studied volar forearm skin. However, $TcPO_2$ measurements at 37°C were extremely low at this site at 1–5 mmHg. In our experience, baseline measurements less than 5 mmHg are not reliable. Therefore, we studied the infraclavicular area, where readings proved generally to be higher than the forearm.

We were unable to study the direct effect of the ointment on the area of skin to which it was applied. Although topical nitroglycerin applied inside the sensor ring raised $TcPO_2$ dramatically at that site, similar though less marked results were obtained with petrolatum. It is unlikely that petrolatum would have an immediate effect on blood flow and oxygen delivery, and we suspect electrode interference as the cause of this artifactual rise in $TcPO_2$. It is also possible that the nitroglycerin vehicle or petrolatum caused small leaks between the skin and sensor rings, which would increase $TcPO_2$. We believe that nitroglycerin may have increased $TcPO_2$ at the exact site where it was applied, but this was not testable with our methodology.

We did find, however, that a 'steal' phenomenon occurs in skin sites immediately adjacent to or up to several centimetres away from the application of nitroglycerin. As each sensor ring is 3·0 cm in diameter and the distance between each ring was 1·0 cm, the nitroglycerin-mediated $TcPO_2$ shunt is observed up to 9·0 cm away from the site of ointment application. The $TcPO_2$ steal was marked when liberal amounts of nitroglycerin ointment were applied, as may occur in the clinical situation. When a smaller quantity of nitroglycerin was used, the steal effect was less marked. Thus, the $TcPO_2$ steal we have demonstrated may be dose-dependent. $TcPO_2$ steal also occurred when the nitroglycerin was applied directly inside sensor A (Fig. 4).

We do not know whether topical nitroglycerin can heal chronic ischaemic ulcers. Some published reports, though anecdotal, suggest that it may help, but we advise caution in its use for these ulcers. Such ulcerations are likely to be in a delicate balance of blood flow and oxygen delivery, which nitroglycerin could shunt from more ischaemic areas to the healthier microvascular bed adjacent to or underlying the ulceration. There is a need for prospective studies of topical nitroglycerin in chronic skin ulcers with concomitant measurements of blood flow and TcPO$_2$.

References

1 Vatner SF, Pagani M, Rutherford JD *et al.* Effects of nitroglycerin on cardiac function and regional blood flow distribution in conscious dogs. *Am J Physiol* 1978; **234:** H244–H52.

2 Taylor SH. The role of transdermal nitroglycerin in the treatment of coronary heart disease. *Am Heart J* 1986; **112:** 197–207.

3 Falanga V, Moosa HH, Nemeth AJ *et al.* Dermal pericapillary fibrin in venous disease and venous ulceration. *Arch Dermatol* 1987; **123:** 620–3.

4 White RA, Nolan L, Harley D *et al.* Noninvasive evaluation of peripheral vascular disease using transcutaneous oxygen tension. *Am J Surg* 1982; **144:** 68–75.

5 Falstie-Jensen H, Spann E, Brochner-Mortensen, Falstie-Jensen S. The influence of epidermal thickness on transcutaneous oxygen pressure measurements in normal persons. *Scand J Clin Lab Invest* 1988; **48:** 519–23.

6 Wheeland RG, Gilchrist RW Jr, Young CJ Jr. Treatment of ischemic digital ulcers with nitroglycerin ointment. *J Dermatol Surg Oncol* 1983; **9:** 548–51.

7 Rinieri J. Apport de la trinitrine percutanee dans le traitement des ulceres rebelles. *Phlebologie* 1987; **40:** 955–8.

8 Testa R, Biagini A, Michelassi C *et al.* Improvement of walking distance in patients with intermittent claudication by chronic local therapy with isosorbide dinitrate ointment. *Angiology* 1988; **39:** 1–7.

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Absorption of hydrocortisone from the skin reservoir in atopic dermatitis

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Summary Percutaneous absorption of hydrocortisone was measured in four children and three adults with atopic dermatitis after the application of 1% hydrocortisone cream and again 12 h after the application of a moisturizer containing 80% water and 5% propylene glycol to the same areas. A significant increase in the level of the plasma cortisol was observed after both applications and these levels were still elevated at 24 h. Topically applied hydrocortisone, stored in eczematous skin, could be released from this reservoir by a moisturizer containing propylene glycol.

The stratum corneum retains topically applied substances and can act as a reservoir. This was first demonstrated for a glucocorticosteroid using a modified vasoconstrictor test.[1] In healthy skin, the reservoir of hydrocortisone may last for several days[2] but little is known of how the reservoir is influenced by eczematous dermatitis. The present study investigated the reservoir function of the skin by measuring the percutaneous absorption of hydrocortisone in patients with atopic dermatitis.

Methods

Patients

Percutaneous absorption of hydrocortisone was studied in four children and three adult patients admitted to hospital with an exacerbation of their atopic dermatitis. Their ages varied from 4 months to 39 years. At the time of the absorption tests, dermatitis was scored by grading the erythema and excoriations separately: 0, none; 1, mild; 2, moderate; 3, severe. The sum of these scores ranged from 3 to 5 (median 4). The extent of dermatitis, as assessed by a burn assessment chart ranged from 60 to 90% (median 90%) (Table 1). The study protocol was approved by an ethical committee. All patients or their parents gave their informed consent.

Plasma cortisol determination

The plasma cortisol levels were determined by [^{125}I]-radioimmunoassay (Farmos Diagnostica, Turku, Finland). In this test the cross-reactivity with dexamethasone was less than 0·005%.

Absorption test

The percutaneous absorption of hydrocortisone was measured in the acute phase of dermatitis using a 24-[3] or 28-h absorption test. Patients were hospitalized and topical glucocorticosteroid therapy withheld for at least 36 h preceding the test. Cortisol secretion was first suppressed by oral dexamethasone.[3] The dose of dexamethasone was 0·25 mg for a body surface area of <0·5 m^2, 0·5 mg for 0·5–0·99 m^2, 0·75 mg for 1·0–1·49 m^2 and 1 mg for a body surface area of >1·5 m^2. Dexamethasone was given at 19.00, 01.00, and 07.00 prior to taking the first blood sample, and thereafter every 6 h. The highest acceptable plasma cortisol level at the beginning of the test, i.e. the lower limit of basal cortisol was 200 nmol/l.[3] No side-effects of dexamethasone were noted. The blood samples for cortisol determination were taken through an intravenous plastic cannula in the morning before the application of 1% hydrocortisone cream at 08.00–09.00 and at 2, 4, 12, 14, 16 and 24 h afterwards. In adults, blood samples were also taken at 26 and 28 h after the application. The amount of 1% hydrocortisone cream needed for topical treatment was recorded for each child (range 33–59 g) and 50 or 75 g was used on the adults. The proprietary cream base contained white petroleum jelly, macrogol 400, cetostearol, cetomacrogol 1000 and methyl parahydroxybenzoate with 60% water. The same amount of moisture cream containing 80% water, 5% propylene glycol, vegetable oil, cetostearyl alcohol, polyethylene glycol monoesterase and diesterase and parabens, was applied immediately after taking the 12-h blood sample. In the three adults, the same amount of this cream was also applied immediately after the 24-h sample. After the

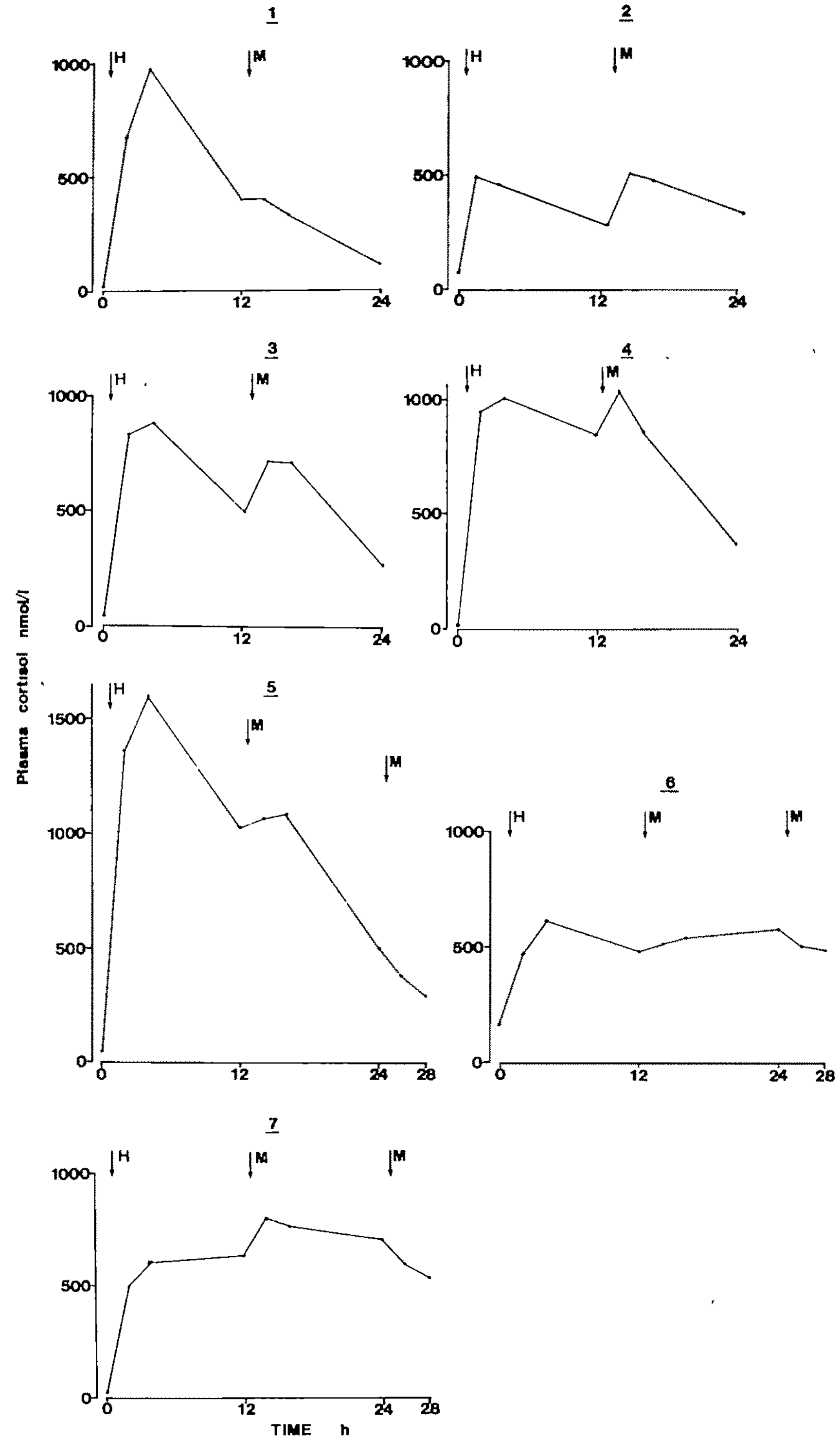

Figure 1. Hydrocortisone absorption curves in seven patients with atopic dermatitis Hydrocortisone cream (H), 1%, was applied immediately after the basal sample (time 0). A moisturizing cream (M) was applied immediately after the 12-h sample. In Patients 5, 6 and 7, the moisturizer was also applied immediately after the 24-h sample. The patients are numbered according to age (No 1-7). 4 months, 8 months, 3, 7, 19, 20 and 39 years

Table 1. Clinical details of patients

Patient	Sex/Age	Covered by dermatitis (%)	Scores for		Amount of cream (g) used in one application
			Erythema	Excoriations	
1	M/4 months	80	2	2	33
2	M/8 months	60	2	1	36
3	F/3 years	90	2	3	59
4	M/7 years	90	3	2	41
5	F/19 years	90	3	2	50
6	F/20 years	90	2	2	75
7	M/39 years	90	3	1	75

applications, the patients wore loose cotton pyjamas. No occlusion was used.

Statistical analysis

The data are presented for each individual. Statistical comparisons were performed using Friedman's non-parametric analysis of variance.[4]

Results

The effect of the adrenocortical suppression caused by dexamethasone was seen in the subnormal (<200 nmol/l) morning basal levels of plasma cortisol of all the patients (range 19–163 nmol/l). The application of 1% hydrocortisone cream was followed by a rise in levels of plasma cortisol within 4 h (Fig. 1). Plasma cortisol levels had declined at 12 h. The application of the moisture cream immediately after taking the 12-h blood sample was followed by a rise in levels of plasma cortisol within 4 h. A further decline in plasma cortisol was observed at 24 h (Fig. 1). The changes between the 0-, 4-, 12-, 16- and 24-h values were significant ($P = 0{\cdot}0005$). There was no rise in levels of plasma cortisol in the three adults after the application of the moisturizer immediately after taking the 24-h blood sample (Fig. 1).

Discussion

Our study confirms previous findings that topically applied hydrocortisone may be absorbed at high levels in severe and widespread dermatitis.[3,5] The present findings also show that topically applied hydrocortisone is stored in the eczematous skin. Twelve hours after the application of hydrocortisone, but not at 24 h, the absorption of hydrocortisone from this reservoir could be significantly increased by the application of a moisturizer containing 80% water and 5% propylene glycol. Propylene glycol is known to increase the penetration of the skin by hydrocortisone from an aqueous hydrophilic ointment.[6]

Because of the passive diffusion of hydrocortisone through the skin, the plasma levels of cortisol reflect the concentrations of the drug in the dermis and viable epidermis, where the pharmacological response is evoked. Elevated plasma levels of hydrocortisone 24 h after application suggest that a single daily application provides enough hydrocortisone for 24 h in the topical hydrocortisone therapy of atopic dermatitis. The absorption may be aided after 12 h by applying a moisturizing cream.

Acknowledgments

I am grateful to the late Dr Osmo P.Salo, former head of the Department, for collaboration, and to Professor Kimmo Mustakallio and Professor Matti Hannuksela for their helpful comments. The study was supported by the Orion Corporation, Helsinki, Finland.

References

1 Vickers CFH. Existence of reservoir in the stratum corneum *Arch Dermatol* 1963; 88: 20–3.

2 Stoughton RB. Dimethylsulfoxide induction of a steroid reservoir in human skin. *Arch Dermatol* 1965; 91: 657–60.

3 Turpeinen M, Salo OP, Leisti S. Effect of percutaneous absorption of hydrocortisone on adrenocortical responsiveness in infants with severe skin disease. *Br J Dermatol* 1986; 115: 475–84

4 Dixon WJ *BMDP Statistical Software*. University of California Press, Berkeley, CA, 1988

5 Turpeinen M. Influence of age and severity of dermatitis on the percutaneous absorption of hydrocortisone in children. *Br J Dermatol* 1988; 118: 517–22.

6 Zesch A, Schaeffer H. Penetration of radioactive hydrocortisone in human skin from various ointment bases. II. *In vivo* experiments. *Arch Dermatol Forch* 1975; 252: 245–56.

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Isotretinoin treatment alters steroid metabolism in women with acne

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Summary

The effect of isotretinoin (Roaccutane) on serum steroids and urinary steroid metabolites was investigated in seven female patients receiving the drug for treatment of severe acne over a 16-week period. Serum concentrations of dehydroepiandrosterone sulphate (DHAS), androstenedione (A2), and free androgen index (FAI) were not significantly altered. There was a significant fall in testosterone during treatment and a significant reduction in the 24 h urinary excretion of androsterone, tetrahydrocortisone (THE) and tetrahydrocortisol (THF) from week 8 onwards and for aetiocholanolone and allo-THF from week 12 ($P<0{\cdot}05$). Although pretreatment levels of urinary steroid metabolites were not abnormal, the ratios of the $5\alpha/5\beta$ metabolites (androsterone:aetiocholanolone and allo-THF:THF) were at the upper limit of the reference range and were lowered after treatment, suggesting that 5α-reductase activity is sensitive to isotretinoin.

Isotretinoin suppresses sebaceous gland activity by blocking the differentiation of basal cells into sebocytes.[1] The exact mechanism is not known but because these glands are androgen-responsive the effects of isotretinoin on androgens have been investigated. A number of studies suggest that sebaceous gland inhibition is not hormonally related but may cause secondary changes in the activity of the skin steroid 5α-reductase.[2] To obtain more information on the response of steroid 5α-reduction to treatment with isotretinoin we measured urinary steroid metabolites by capillary column gas liquid chromatography and serum androgen levels (testosterone, androstenedione (A2), and dehydroepiandrosterone sulphate (DHAS)) by radioimmunoassay during the course of 16 weeks of therapy with isotretinoin (Roaccutane).

Methods

Subjects

Seven women, median age 26·7 years (range 18–44 years) with persistent acne that was non-responsive to antibiotics were studied. All were using barrier methods of contraception and had a normal menstrual pattern with regular cycles of between 21 and 35 days. There was no evidence of hirsutism, hair loss or virilism. Pretreatment liver function and fasting serum lipids were normal and remained so during treatment. All seven women were given a 16-week course of isotretinoin (Roaccutane, Roche Products Ltd) at a dose of 1 mg/kg/body weight per day. The mean total acne grade[3] before starting was 3·5 (95% confidence interval, 0·3–6·6) with a mean facial grade of 1·4 (0·1–2·7). Twenty-four-hour urine samples were collected immediately before starting the drug and at 4-weekly intervals thereafter. Facial and total acne were also scored at monthly intervals.[3]

Hormone analysis

Sex hormone binding globulin (SHBG) was measured by time-resolved fluoroimmunoassay ('Delfia' SHBG kits, Pharmacia). Testosterone, DHAS and A2 were measured by conventional 'in house' radioimmunoassays. Derived free testosterone levels were calculated from the total testosterone and SHBG according to the method of Nanjee and Wheeler.[4] Urinary steroid profiles analysis was performed by capillary column chromatography by a method similar to that of Bevan *et al.*[5] Separation of steroid methyloxime-trimethylsilyl ether derivatives was carried out on a model 438 gas chromatograph (Packer-Becker BV, Delft, The Netherlands) fitted with a 'Flexsil' 25 m × 0·32 mm capillary column coated with OV-1 (Phase-Sep, Queensferry, Clwyd). Measurements were made of the most abundant androgen metabolites

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(androsterone, aetiocholanolone) and glucocorticoid metabolites (tetrahydrocortisone (THE), tetrahydrocortisol (THF), and allo-tetrahydrocortisol (allo-THF)). Ratios of $5\alpha:5\beta$ pairs (i.e. androsterone:aetiocholanolone and allo-THF:THF ratios) were calculated to provide an index of exogenous 5α-reductase activity.

Statistics

Results are presented as mean ± standard error of the mean (95% confidence interval). Statistical analyses were by the non-parametric tests of analysis of variance and by the Wilcoxon sign-rank test.

Results

Acne was virtually abolished in all patients by the end of the 4-month treatment period; the mean total acne score was 0·25 (−0·05–0·5) and the mean facial acne score was 0·07 (0·02–0·12). All patients experienced the common side-effects of isotretinoin (dry lips and eyes) but remained well throughout the treatment period and experienced no significant alteration of their liver function tests or fasting serum lipids. Menstrual patterns did not change.

The effects of isotretinoin treatment on serum androgens and SHBG concentrations are shown in Table 1. The mean baseline concentrations of testosterone, DHAS, and SHBG were normal while a modest elevation in A2 was observed (due to an abnormally high A2 level of 16·1 nmol/l in one patient suspected of suffering from polycystic ovarian disease; others were all within the normal range 0·6–8·8 nmol/l). The mean androstenedione concentration fell during treatment; this fall was not significant and was largely due to normalization of serum A2 in the patient with an elevated pretreatment level. Over the 16-week treatment period there was a significant fall in serum testosterone at weeks 8 and 16 ($P<0{\cdot}05$).

Table 1. Serum steroid concentrations (mean ± SEM) of seven female patients with severe acne during a 16-week course of isotretinoin therapy (1 mg/kg/day) *$P<0{\cdot}05$ compared with baseline

		Weeks			
Hormone (reference range)	Baseline	4	8	12	16
Testosterone (1–3·2 nmol/l)	1·9±0·3	1·7±0·2	1·2±0·1*	1·5±0·2	1·3±0·1*
Dehydroepiandrosterone sulphate (2–11 μmol/l)	7·4±1·8	10·7±3·8	7·7±1·6	7·9±2·0	7·2±1·7
Sex hormone binding globulin (30–120 nmol/l)	79±19	71±17	71±21	70±21	71±20
Androstenedione (nmol/l) (0·6–8·8 nmol/l)	9·4±2·3	8·1±1·4	7·5±1·1	8·9±2·4	7·4±1·8
Free androgen index (0·8±5·2)	3·4±1·4	2·8±0·7	2·5±0·6	3·5±1·5	2·4±1·1

Table 2. Total 24 hour urinary excretion of steroids (mean ± SEM, μg/24 h) of seven female patients with severe acne during a 16-week course of isotretinoin therapy (1 mg/kg day). *$P<0{\cdot}05$ compared with baseline

		Weeks			
Hormone (reference range)	Baseline	4	8	12	16
Androsterone (1029±457 μg)	1205±286	1090±226	596±121*	538±664*	664±194*
Aetiocholanolone (1335±525 μg)	1086±241	1175±288	730±157	609±207*	740±50*
Tetrahydrocortisone (THE) (3221±946 μg)	2708±527	2855±759	1480±234*	1553±322	1587±292*
Tetrahydrocortisol (THF) (1617±525 μg)	1331±218	1298±246	778±161*	835±214	750±133*
Allo-Tetrahydrocortisol (allo-THF) (1008±491 μg)	1293±302	1212±439	572±184	450±101*	548±117*

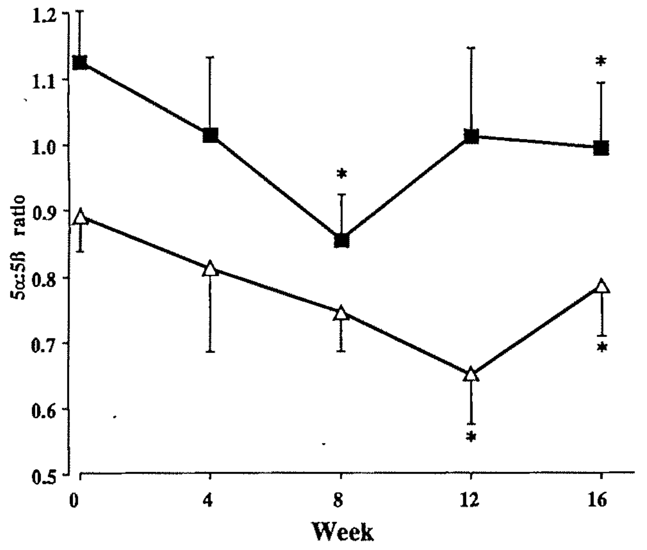

Figure 1. Ratio of 5α:5β urinary steroid metabolites (mean±SEM) in seven female patients with severe acne during a 16-week course of isotretinoin therapy (1 mg/kg/day) (* $P<0{\cdot}05$ from baseline). ■, androsterone:aetiocholanolone; △, allo-THF:THF.

Total 24-hour excretion of the selected steroid metabolites are shown in Table 2: the baseline concentrations of the patients were within the expected limits for normal women. Over the study period, a marked reduction in urinary steroid metabolites was observed with significant falls of androsterone, THE and THF from week 8 onwards and aetiocholanolone and allo-THF from week 12 ($P<0{\cdot}005$).

Baseline ratios of both androsterone:aetiocholanolone (1·12±0·09) and allo-THF:THF (0·89±0·05) were at the upper limit of normal (normal mean values 0·65±0·2, range 0·35–1·15 and 0·62±0·2, range 0·2–1·0, respectively). These high ratios fell significantly during treatment (Fig. 1).

Discussion

A number of studies suggest that sebaceous gland involution induced by isotretinoin is not androgen-mediated. This conclusion is based on the lack of any dramatic effect of the drug on circulating precursor androgens. Lookingbill *et al.*[2] found that serum androgens testosterone, DHA and DHAS were not altered by treatment although an increase in free testosterone was found and Matsuoka *et al.*[6] found no change in circulating concentrations of cortisol, androstenedione, DHA and DHAS. Some of these findings are confirmed in this study where no significant change in DHAS, androstenedione and free androgen index occurred on treatment. We did however find a small but significant fall in the serum level of testosterone.

A more significant inhibitory effect was observed[2] on production of 5α androgens (dihydrotestosterone and 3α-androstenediol glucuronide in women; 3α-androstenediol glucuronide in men) and this was thought to be due to the reduction in size of the sebaceous gland. We have previously reported significant but small reductions in serum dihydrotestosterone in women on isotretinoin.[7] Whether or not isotretinoin has a direct effect on steroid 5α-reduction *in vivo* has, until now, been uncertain. *In vitro* studies of testosterone metabolism in hamster flank organ showed lack of inhibition of 5α-reductase by isotretinoin[8] but in female rats isotretinoin treatment caused a decrease in steroid 5α-reductase activity in both skin and liver.[9] In cultured human prostatic cells 5α-reductase can be inhibited by retinoic acid through competitive inhibition of cofactor action (NADPH)[10] yet in men circulating dihydrotestosterone is unchanged throughout isotretinoin therapy.[2]

In the present study marked reductions in the major androgen and glucocorticoid metabolites were found at the same time as decreased levels of 5α-reduction. 5α-reduction of androgens occurs in the peripheral tissues as well as the liver while glucocorticoids are mainly 5α-reduced in liver. It is therefore unlikely that these changes can be accounted for solely by drug-induced reduction in the size of the sebaceous gland and a direct effect on liver 5α-reduction is postulated. A clue as to how these aspects of steroid metabolism are related is given by recent reports on steroid metabolism in women with hirsutism and polycystic ovarian disease.[11–12] In such patients it is postulated that increased 5α-reduction in the liver enhances hepatic cortisol metabolism and circulating cortisol concentrations are maintained within normal limits by increasing the secretion of corticotrophin which simulates adrenal production of both glucocorticoids and androgens. It may be that, by inhibiting 5α-reduction within the liver, isotretinoin reduces cortisol excretion, and thereby reduces corticotrophin secretion, the stimulus for adrenal steroid production. Alterations in steroid metabolism of a similar nature occur in patients suffering from acute intermittent porphyria but in this case 5α-reduction may be secondary to endogenous rather than exogenous factors.[13]

Further work is required to verify and extend these findings. It is of course possible that the observed effects on steroid metabolism are related to the as yet unknown mechanism of action of retinoids in controlling cell differentiation.

References

1 Landthaler M, Kummermehr J, Wagner A, Plewig G. Inhibitory effects of 13-*cis*-retinoic acid in human sebaceous glands. *Arch Dermatol Res* 1980; **269**: 297–309.

2 Lookingbill DP, Demers LM, Tigelaar RE, Shalita AR. Effect of isotretinoin on serum levels of precursor and peripherally derived androgens in patients with acne. *Arch Dermatol* 1988; **124**: 540–3.

3 Burke BM, Cunliffe WJ. The assessment of acne vulgaris: the Leeds technique. *Br J Dermatol* 1984; **111**: 83–92.

4 Nanjee MN, Wheeler MJ. Plasma free testosterone—is an index sufficient? *Ann Clin Biochem* 1985; **22**: 387–90.

5 Bevan BR, Savvas M, Jenkins JM *et al.* Abnormal steroid excretion in gestational trophoblastic disease complicated by ovarian theca-lutein cysts. *J Clin Pathol* 1986; **39**: 627–34.

6 Matsuoka LY, Wortsman J, Lifrak ET *et al.* Effect of isotretinoin in acne is not mediated by adrenal androgens. *J Am Acad Dermatol* 1989; **20**: 128–9.

7 Simpson NB, Cunliffe WJ, Rademaker M *et al.* The sebaceous gland as a source of androgen—an hypothesis. In: *Acne and Related Disorders.* (Marks R, Plewig G eds). London: M Dunitz Ltd, 1989.

8 Gomez EC, Moskowitz RJ. Effect of 13-cis-retinoic acid on hamster flank organ. *J Invest Dermatol* 1980; **74**: 392–7.

9 Graef V, Ritter B, Goerz G. Effect of synthetic retinoids on 5α-reductases in rat liver and skin. *Proc of 8th Int Cong of Endocrinology* (July 1988, Kyoto, Japan), p 423.

10 Halgunset J, Sunde A, Lundmo PI. Retinoic acid (RA): an inhibitor of 5α-reductase in human prostatic cancer cells. *J Steroid Biochem* 1987; **28**: 731–6.

11 Buchanan LM, Wallace AM, Thomson J. Abnormal steroid metabolism in hirsute women. *Scott Med J* 1990; **35**: 155.

12 Stewart PM, Shackleton CHL, Beastall GH, Edwards CRW. 5α-reductase activity in polycystic ovary syndrome. *Lancet* 1990; **335**: 431–3.

13 Herrick AL, Wallace AM, McColl KEL *et al.* Association of elevated sex hormone binding globulin with abnormal steroid metabolism in acute intermittant porphyria *J Endocrinol* 1988; **Suppl 119**: 114.

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PUVA treatment of psoriasis in the United Kingdom

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Summary Information concerning the operation of 134 PUVA units in 96 dermatology centres in the U.K. was collected by a postal questionnaire. The most common protocol was to give PUVA three times weekly in the treatment of psoriasis, and to choose the initial dose of UVA according to skin type. However, the initial UVA dose encompassed a range of five or more within each skin type.

Since its introduction in the 1970s[1] oral psoralen photochemotherapy (PUVA) has become an established second-line treatment for psoriasis. Certain components of the treatment protocol appear to have been standardized, such as the dose of psoralen and the timing of UVA exposure after psoralen administration.[2] However, other equally important components, such as the method of choosing the initial dose of UVA, and the frequency of treatment, are known to show considerable variation between centres. In particular, two treatment protocols have been widely described: that used in the United States[3] in which the starting dose of UVA is chosen according to skin type and treatment is given two or three times each week; and that said to be used in Europe[4] in which the minimal phototoxic dose is determined before treatment and which is then given four times each week. It has been suggested[5,6] that differences in these treatment protocols may account for the variation in incidence of non-melanoma skin cancer found in follow-up studies of PUVA treated patients.[7,8]

There are no published data concerning the methodology of PUVA treatment employed in the U.K. We now report the results of a postal questionnaire designed to determine the location and make of PUVA units, and their operation in the treatment of psoriasis.

Methods

In November 1989 a questionnaire was sent to 170 consultant dermatologists in the U.K. Names and addresses were obtained from the office of the British Association of Dermatologists and, to avoid duplication of responses, the questionnaire was sent to only one representative dermatologist at each hospital centre.

The questionnaire sought information concerning the number of 'whole body' PUVA units in use at each centre, their location within the hospital and the manufacturer of the PUVA equipment. Three further questions concerned the frequency of treatment and the method of choosing the initial dose of UVA.

Results

Of the 170 questionnaires that were sent, 132 (78%) were returned giving information on 134 PUVA units located in 96 different dermatology centres. In 36 cases PUVA treatment was not available locally and five replies indicated that patients were sent elsewhere for treatment. The majority (67%) of the 96 centres with PUVA used one 'whole body' unit, 28% used two and 5% used three. PUVA treatment was performed in the out-

Table 1. The manufacturers and numbers of PUVA units in the U.K.

Unit	Manufacturer	Number of units
PUVA series (e.g. PUVA 6002)	H Waldmann GmbH. (Germany)	83
London Photochemotherapy Apparatus	Previously Rank Stanley Cox, then Duffield Medical Ltd (U.K.) No longer manufactured	24
Blacklight Series III	Coast-Air Ltd (U.K.)	14
Dermalight (previously called 'Bluelight')	Dr Hönle (Germany)	5
Photochemotherapy Apparatus	Canterbury Instruments (U.K.)	1
Other	Unknown manufacturer or 'home made'.	7

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Table 2. The doses of UVA given at the start of a course of PUVA to clear psoriasis. Values are the percentage of the 76 treatment centres that choose the starting dose of UVA according to skin type

	Starting dose of UVA (J/cm^2)									
Skin type	<0·5	0·5	1·0	1·5	2·0	2·5	3·0	3·5	4·0	4·5
I	3	62	13	20	2	0	0	0	0	0
II	0	34	38	3	4	21	0	0	0	0
III	0	7	34	25	7	2	6	19	0	0
IV	0	8	15	15	23	6	8	6	1	18

patient area in 47% of centres, in the dermatology ward in 23% and in the physiotherapy department in 30%. The number of PUVA units according to the manufacturer is shown in Table 1.

For a course of treatment to clear psoriasis, PUVA was given twice a week in 24 centres, three times a week in 67 centres and four times a week in one centre. The question concerning frequency of treatment was not answered in responses from four centres. At the start of the course a fixed dose of UVA, irrespective of skin type, was used in 15 centres. The starting dose was chosen after measurement of the minimal phototoxic dose in five centres. In the remaining 76 cases, the starting dose of UVA was based upon the skin type of the individual patient. The variation in doses chosen for patients with different skin types is shown in Table 2.

Discussion

The response rate of 78% ensures that the information received concerning PUVA treatment in 96 different dermatology centres is representative of PUVA treatment in the U.K. PUVA treatment was not available locally in 28% of centres and many of the returned questionnaires indicated that unsuccessful requests for funding had been made. That dermatology centres without PUVA form such a small minority may be useful information in future applications for funding.

In the U.K., UVB phototherapy of psoriasis is most commonly performed within physiotherapy departments (B.L.Diffey, A.F.McKinlay, unpublished data), whereas it appears from our results that PUVA treatment is performed largely within dermatology departments. This may reflect the need or desirability for closer dermatological supervision of PUVA treatment than UVB phototherapy.

The question concerning the manufacturer of PUVA equipment was included principally to determine the prevalence of units that contained filtered metal halide lamps (Dermalight) as opposed to conventional UVA fluorescent lamps (all other manufacturers). Direct comparisons of therapeutic response to these two UVA sources have not been made but recent work suggests that the bias towards longer-wavelength UVA, as obtained from metal halide lamps, may be less effective than conventional fluorescent sources.[9] It is clear that the majority of PUVA units used in this country are manufactured by the German company Waldmann GmbH. Despite the fact that PUVA units were being manufactured in the U.K. by the mid 1970s, British industry has not secured more than a small share of the market.

The early protagonists of PUVA therapy stressed the importance of careful dosimetry and the need to base starting doses of UVA on either skin type or the results of phototesting.[10] It is surprising, therefore, that the starting doses of UVA that were reported in our survey encompass a range of five or more within each skin type. Intercomparisons of UVA dosimetry in the U.K.,[11] Belgium[12] and France[13] have shown a wide range in the accuracy of UVA measurement in PUVA centres, such that the irradiance measured by any single UVA meter may be between 0·5 and 1·5 times the true irradiance. It is possible that if the accuracies of the UVA meters used in each of the 96 centres represented here were known and corrected for, the real range in starting doses might be less.

It is clear that in the U.K. there is great variation in the methodology of PUVA treatment for psoriasis. The majority of treatment centres base the starting dose of UVA on skin type, as in the protocol originally devised in the United States.[3] However, interpretation of studies of long-term hazards of PUVA therapy may be difficult because of the apparently large variation in choice of UVA dose between centres. Clinical trials are still needed to identify the PUVA treatment protocol that allows the most efficient clearance of psoriasis.

Acknowledgment

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References

1 Parrish JA, Fitzpatrick TB, Tanenbaum L, Pathak MA. Photochemotherapy of psoriasis with oral methoxalen and longwave ultraviolet light. *New Engl J Med* 1974; **291**: 1207–11.

2 Frain-Bell W. *Cutaneous Photobiology*. Oxford: Oxford University Press, 1985; 181.

3 Melski JW, Tanenbaum L, Parrish JA *et al* Oral methoxalen photochemotherapy for the treatment of psoriasis. a cooperative clinical trial. *J Invest Dermatol* 1977; **68:** 328–35.

4 Hensler T, Wolff K, Honigsmann H, Christophers E. Oral 8-methoxypsoralen photochemotherapy of psoriasis. The European PUVA study: a cooperative study among 18 European centres. *Lancet* 1981; **i:** 853–7.

5 Gupta AK, Anderson TF. Psoralen photochemotherapy. *J Am Acad Dermatol* 1987; **17:** 703–34.

6 Gibbs NK, Honigsmann H, Young AR. PUVA treatment strategies and cancer risk. *Lancet* 1986; **i:** 150–1.

7 Henseler T, Christophers E, Honigsmann H, Wolff K. Skin tumors in the European PUVA study. *J Am Acad Dermatol* 1987; **16:** 108–16.

8 Stern RS, Laird N, Melski J *et al.* Cutaneous squamous-cell carcinoma in patients treated with PUVA. *N Engl J Med* 1984; **310:** 1156–61.

9 Farr PM, Diffey BL, Higgins EM, Matthews JNS. The action spectrum between 320 and 400 nm for clearance of psoriasis by psoralen photochemotherapy. *Br J Dermatol* 1991; **124:** (in press).

10 Wolff K, Gschnait F, Honigsmann H *et al* Phototesting and dosimetry for photochemotherapy. *Br J Dermatol* 1977, **96:** 1–14.

11 Diffey BL, Challoner AVJ, Key PJ A survey of the ultraviolet radiation emissions of photochemotherapy units. *Br J Dermatol* 1980; **102:** 301–6

12 Diffey BL, Roelandts R. Status of ultraviolet A dosimetry in methoxalen plus ultraviolet A therapy. *J Am Acad Dermatol* 1986; **15:** 1209–13.

13 Roelandts R, Diffey BL, Bocquet JL. Une dosimetrie UVA precise en photobiologie et un photodermatologie est-elle une illusion? *Ann Dermatol Venereol* 1988; **115:** 1261–4.

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Efficacy and safety of CD 271 alcoholic gels in the topical treatment of acne vulgaris

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Summary

CD 271, a naphthoic acid, is a powerful modulator of epidermal differentiation. This double-blind, randomized study compared the efficacy and safety of two concentrations (0·03% w/w and 0·1% w/w) of CD 271 alcoholic gel, with 0·025% w/w tretinoin gel in 72 male patients with acne vulgaris over a period of 12 weeks. Efficacy was measured by counting facial inflammatory and non-inflammatory lesions and by grading the severity of the acne at each visit. Skin tolerance was assessed with subjective symptoms, such as burning and pruritus, as well as clinical assessment of erythema, dryness and scaling on the treated areas. The alcoholic 0·1% CD 271 gel was as effective as 0·025% tretinoin gel in reducing total comedone counts (83% reduction for both products after 12 weeks' treatment). The reduction in the number of inflammatory lesions and the total number of acne lesions were significantly greater with 0·1% CD 271 gel than with tretinoin gel (69% and 79% for 0·1% CD 271, 50% and 73% for tretinoin gel, respectively, $P<0{\cdot}05$). All three treatments were well tolerated and there were no changes in any major blood parameters. No CD 271 could be detected in blood plasma at the end of the study (detection limit = 1 ng/ml).

Acne vulgaris is a multifactorial disease where androgens, sebum and micro-organisms (*Propionibacterium acnes*) are involved in the production of lesions. All-*trans* retinoic acid has been used for more than 20 years in the topical treatment of acne vulgaris and more recently topical 13-*cis* retinoic acid was also found to be effective.[1] CD 271 is a synthetic naphthoic acid derivative and its anti-inflammatory and comedolytic activity in animal models[2–4] indicated that it would be a promising compound for the topical treatment of acne. Unpublished vehicle-controlled clinical studies with CD 271 on acne patients showed a significant therapeutic efficacy. The purpose of this clinical study was to compare the efficacy and safety of 0·03% and 0·1% w/w CD 271 in a gel formulation to 0·025% tretinoin gel in the treatment of mild to moderate facial acne.

Methods

This study was carried out at the Warsaw Dermatological Clinic from October 1987 to March 1988. The protocol of the trial was approved by the local Ethical Committee.

Seventy-two male patients with facial acne vulgaris, grade 1–3,[5] were included in the study and each gave written informed consent. Because of insufficient toxicity data on women at that time, no female patients were recruited for the study. The study was double-blind and randomized and compared three parallel groups of 24 patients receiving either 0·03% or 0·1% CD 271 alcoholic gel or 0·025% tretinoin gel. Identical tubes were used for all three preparations.

The patients' mean age was 18·7 years (16–23 years). Before starting the study, the patients had not used any local acne treatment for at least 4 weeks or any systemic retinoid therapy over the previous 6 months. Patients were randomly assigned to treatment groups and the three study groups were found to be homogeneous with regard to age, skin type and initial lesion counts. The patients were instructed to apply the medication to their face each night, at least 15 min after washing and at least 1 h before retiring. The use of other topical or oral anti-acne treatment was prohibited during the study. The treatment lasted 12 weeks and clinical examinations were performed at 1, 2, 4, 8 and 12 weeks after the initial visit. The investigator assessed the severity of the facial acne by counting the lesions: closed comedones, open comedones, papules, superficial pustules,

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deep seated pustules, nodules and cysts. An overall clinical assessment was also carried out using the grading method as described by Burke and Cunliffe[5] and using photographs for reference. Side-effects were recorded at each visit and each of the following criteria, erythema, scaling, burning, pruritus and skin dryness, were scored from 0 to 3 (0, none; 1, mild; 2, moderate, 3, severe). At the final visit, each patient was asked to evaluate his response to treatment.

Blood samples were obtained before and at the end of the 12-week treatment period to determine the level of CD 271 in the plasma (detection limit sensitivity: 1 ng/ml) and for a full blood investigation including: platelets, haemoglobin, proteins, electrolytes, urea, creatinine, glucose, bilirubin, AST (SGOT), ALT (SGPT), alkaline phosphatase, cholesterol and triglycerides. For the CD 271 assay, the plasma was frozen at −20°C after separation and later analysed using HPLC with a fluorescence detector.

Statistical analysis

Before analysis the lesion counts were transformed into their square root to normalize their distribution. Analysis of covariance was performed with the baseline value as covariate. Two-tailed tests were performed and for the overall between-treatment comparison, the analysed parameters for each treatment were averaged across the different clinical examinations (weeks 1–12). A *posteriori* calculation showed that a difference of 14% between the CD 271 and 0·025% tretinoin groups for mean per cent reductions in lesion counts at week 12 had a probability of 80% of being detected for non-inflammatory and total lesion counts. For inflammatory lesions, the smallest difference that had an 80% probability of detection was 20%.

Results

Efficacy

The results for non-inflammatory lesions showed that 0·1% CD 271 alcoholic gel was as effective as 0·025% tretinoin in reducing the number of non-inflammatory lesions with an 83% reduction by week 12 (Fig. 1, Table 1). Their efficacy was significantly different from 0·03% CD 271 gel ($P<0{\cdot}01$ and $P<0{\cdot}05$, respectively). Closed comedones were reduced by all three treatments. However, 0·1% CD 271 alcoholic gel was significantly more effective than 0·03% CD 271 alcoholic gel ($P<0{\cdot}01$).

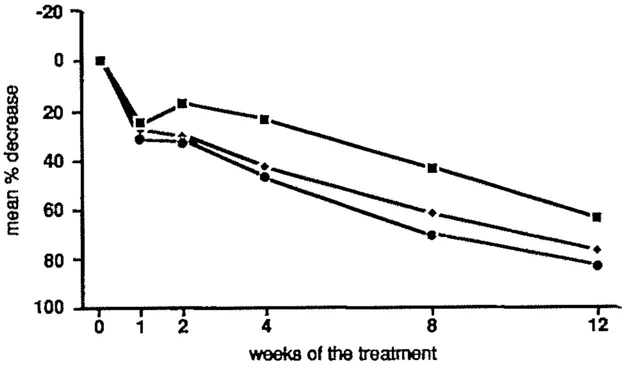

Figure 1. Mean per cent change from baseline in number of non-inflammatory lesions. ◆, 0·025% tretinoin; ■, 0·03% CD 271; ●, 0·1% CD 271.

Tretinoin gel (0·025%) and 0·1% CD 271 alcoholic gel produced identical reductions in the number of closed comedones.

Open comedones were significantly reduced by all three treatments ($P<0{\cdot}01$) and there were no between-group differences. After 3 months of treatment, four patients treated with 0·1% CD 271 gel and three with 0·025% tretinoin gel were completely cleared of non-inflammatory lesions.

Inflammatory lesions were significantly reduced by all three regimens (Fig. 2, Table 1). However, 0·1% CD 271 alcoholic gel was significantly more effective against inflammatory lesions than 0·025% tretinoin gel (69% and 50% reduction at week 12, respectively, $P<0{\cdot}05$). A marked decrease in the inflammatory lesion count was produced after 1 week of treatment with both CD271 concentrations (Fig. 2) with a marked dose-related response. At this early stage of treatment, half the patients treated with 0·025% tretinoin gel had a transient flare-up of their lesions, mainly papules. CD 271 gels 0·1% and 0·03% were significantly more effective than tretinoin on the inflammatory lesions at week 1 ($P<0{\cdot}01$ and $P<0{\cdot}05$, respectively).

The total lesion count was significantly reduced by all three treatments. The total lesion number was significantly more reduced with 0·1% CD 271 alcoholic gel (79% reduction) than with 0·025% tretinoin gel (73% reduction, $P<0{\cdot}05$), and a dose-related response was again observed for CD 271. The global grading method (Table 1) was less sensitive than counting the acne lesions and could not detect any difference between the groups treated with 0·1% CD 271 gel and 0·025% tretinoin gel.

Table 1. Means (±SE) of non-inflammatory and inflammatory lesions and global grades before and after 12 weeks' treatment within each treatment group

Treatment groups	Pretreatment	Post-treatment	Significance of difference between treatments (week 12)
Non-inflammatory lesions			
0·03% CD 271 (n=24)	94·3 (±11·1)	27·6 (±4·6)	0·1% CD >0·03% CD (P<0·05)
0·1% CD 271 (n=24)	113·4 (±14·5)	19·7 (±5·5)	0·1% CD=0·025% T
0·025% Tretinoin (n=24)	99·7 (±15·8)	16·8 (±2·2)	
Inflammatory lesions			
0·03% CD 271 (n=24)	49·8 (±5·0)	21·7 (±2·4)	0·1% CD >0·03% CD (P<0·05)
0·1% CD 271 (n=24)	48·2 (±4·3)	15·0 (±2·1)	0·1% CD >0·025% T (P<0·05)
0·025% Tretinoin (n=24)	41·3 (±3·0)	20·6 (±2·1)	
Global grade			
0·03% CD 271 (n=24)	1·61 (±0·06)	0·80 (±0·10)	0·1% CD >0·03% CD (P<0·05)
0·1% CD 271 (n=24)	1·49 (±0·07)	0·45 (±0·05)	0·1% CD=0·025% T
0·025% Tretinoin (n=24)	1·95 (±0·13)	0·86 (±0·17)	

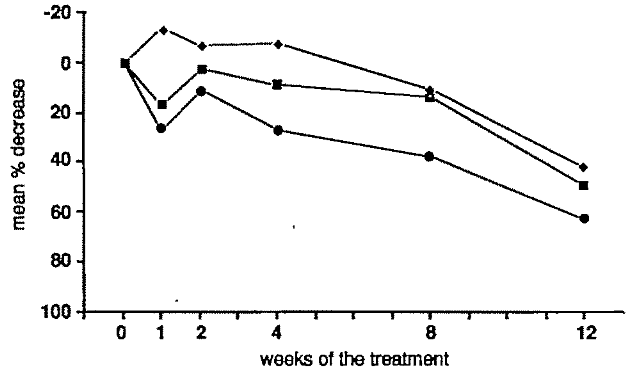

Figure 2. Mean per cent change from baseline in number of inflammatory lesions. ◆, 0·025% tretinoin; ■, 0·03% CD 271, ●, 0·1% CD 271.

Table 2. Mean skin safety scores at week 1 in each treatment group

	0·03% CD 271 (n=24)	0·1% CD 271 (n=24)	0·025% Tretinoin (n=24)
Erythema	1·0±0·1	1·3±0·2	1·0±0·2
Scaling	0·6±0·2	1·3±0·2	0·8±0·1
Pruritus	0·1±0·1	0·1±0·1	0·1±0·1
Burning	1·3±0·1	1·8±0·2	1·4±0·2
Dryness	0·0	0·4±0·1	0·0

Side-effects

All patients in the study completed a 3-month course of treatment. One had severe, but transient erythema with 0·1% CD 271 alcoholic gel and one with 0·025% tretinoin gel. Mean skin safety scores at week 1 (Table 2) show that 0·1% CD 271 provoked more scaling than 0·025% tretinoin gel and this difference was significant throughout the treatment period. In those treated with 0·1% CD 271 there were slightly more cases with burning sensations than in those treated with 0·03% CD 271 and tretinoin. The mean erythema score was low for 0·03% CD 271 and 0·025% tretinoin and slightly more for 0·1% CD 271, but this was not significant. The mean erythema scores declined significantly with time (P<0·01) over the 3-month treatment period.

The subjective symptoms reported by some patients were described as a mild burning immediately after application, rather than persistent burning. This slight discomfort did not result in any of the patients discontinuing treatment. Mean pruritus and skin dryness scores were generally close to 0 at week 1 and varied little during the 3-month treatment period. Thus, the skin safety profiles of 0·03% CD 271 and 0·025% tretinoin were similar, whereas the 0·1% CD 271 tolerance score was slightly higher.

Patient self-assessment

During the final visit, the clinician recorded the patient's opinion of the treatment. This showed a preference for 0·1% CD 271 alcoholic gel. There was no marked difference of opinion between 0·03% CD 271 and 0·025% tretinoin gel.

Systemic safety evaluation

There were no significant abnormalities in the blood investigations carried out during treatment and no significant amounts of CD 271 could be detected in the plasma of patients receiving CD 271 (detection limit of 1 ng/ml) on their last day of treatment.

Discussion

Topical tretinoin has been shown to be effective in reducing comedones in animal models and in acne patients.[4,6] A reduction of inflammation with topical anti-acne compounds can be achieved with antibiotics[7–10] and with isotretinoin.[1] During the preclinical screening, CD 271 showed both potent comedolytic properties using the rhino mouse and moderate to potent anti-inflammatory effects in different *in vitro* and *in vivo* models,[3,4] suggesting the compound would be effective in the treatment of acne. In the present study, 0·1% CD 271 gel was demonstrated to be as effective as 0·025% tretinoin gel in reducing comedones in acne patients, and was shown to be superior to 0·025% tretinoin gel in reducing the number of inflammatory lesions. Furthermore, a dose–response relationship was shown for the two CD 271 concentrations (0·03% and 0·1%).

The skin safety profiles of CD271 and tretinoin were equally acceptable and no patient dropped out of the study because of side-effects during the 3-month treatment period. Additional studies are to be undertaken to further evaluate the safety and efficacy of CD 271. However, the present study suggests that 0·1% CD 271 alcoholic gel may be an effective and safe treatment for mild to moderate acne vulgaris.

Acknowledgments

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References

1 Chalker DK, Lesher JL Jr, Smith JG Jr *et al.* Efficacy of topical isotretinoin 0·05% gel in acne vulgaris: results of a multicenter, double-blind investigation *J Am Acad Dermatol* 1987; **17**: 251–4.

2 Shroot B A strategy for discovery of new retinoid-like substances. In: *Pharmacology of Retinoids in the Skin*. (Reichert U, Shroot B, eds), Vol 3. Basel. Karger 1989, 270.

3 Hensby C, Cavey D, Bouclier M *et al.* The *in vivo* and *in vitro* anti-inflammatory activity of CD 271, a new retinoid-like modulator of cell differentiation. In: *Pharmacology of Retinoids in the Skin* (Reichert U, Shroot B, eds), Vol 3. Basel: Karger 1989; 160.

4 Chatelus A, Caron JC, Shroot B *et al.* Structure-activity relationships between different retinoids using the topical rhino mouse comedolytic model. In: *Pharmacology of Retinoids in the Skin* (Reichert U, Shroot B, eds), Vol 3. Basel: Karger 1989; 144.

5 Burke BM, Cunliffe WJ. The assessment of acne vulgaris: the Leeds technique *Br J Dermatol* 1984; **111**: 83–92.

6 Christiansen J, Holm P, Reymann F. The retinoic acid derivative Ro 11-1430 in acne vulgaris. A controlled multicenter trial against retinoic acid. *Dermatologica* 1977; **154**: 219–27.

7 Gammon WR, Meyer C, Lantis S *et al.* Comparative efficacy of oral erythromycin versus oral tetracycline in the treatment of acne vulgaris. *J Am Acad Dermatol* 1986; **14**: 183–6.

8 Harrison PV. A comparison of doxycycline and minocycline in the treatment of acne vulgaris. *Clin Exp Dermatol* 1988; **13**: 242–4

9 Katsambas A, Towarky A, Stratigos J. Topical clindamycin phosphate compared with oral tetracycline in the treatment of acne vulgaris. *Br J Dermatol* 1987; **116**: 387–91.

10 Leyden JJ, Shalita AR, Saatjian GD, Sefton J. Erythromycin 2% in comparison with clindamycin phosphate 1% solution in acne vulgaris. *J Am Acad Dermatol* 1987; **16**: 822–7.

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Relative efficacy of 335 and 365 nm radiation in photochemotherapy of psoriasis

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Summary

The action spectrum for the induction of phototoxic erythema by treatment with 8-methoxypsoralen (8-MOP) and UVA peaks at around 335 nm. Because earlier investigations reported peak phototoxic activity at 365 nm, we compared the antipsoriatic efficacy of 335 and 365 nm in an oral psoralen photochemotherapy (PUVA) regimen using a monochromator. PUVA with 335 nm was twice as effective as with 365 nm, with respect to both erythemogenicity and the cumulative dose required for clearing psoriasis. However, 335 and 365 nm were equally effective if delivered in equal erythema doses. It appears that in human skin the antipsoriatic activity of 8-MOP parallels its erythemogenicity.

Radiation sources in psoralen photochemotherapy (PUVA) are usually employed at peak wavelengths of between 350 and 355 nm and there is abundant evidence that this band is effective in the treatment of psoriasis. The rationale for using this particular band of UVA is based on early psoralen photosensitization experiments which have led to the assumption that the biological action spectrum ranges between 340 and 380 nm with a maximum at 365 nm.[1,2] Later studies revealed maximum erythema effectiveness in psoralen-sensitized guinea-pigs in the range of 330–350 nm.[3,4] More recently, an action spectrum for phototoxic erythema with topical 8-methoxypsoralen (8-MOP) was established which showed peak effectiveness at 330 nm.[5] Sunburn cell formation in mammalian epidermis[6] and phototumorigenesis[7] induced by 8-MOP and UVA in hairless mice peak at 335 nm. In addition, maximum inhibition of epidermal DNA synthesis was demonstrated to occur at 335 nm.[8] Because it is commonly assumed that the suppression of DNA synthesis and cell division is decisive in suppressing the epidermal hyperproliferative stimulus in psoriasis,[9–12] we compared the antipsoriatic efficacy of PUVA at 365 and 335 nm.

Methods

Patients

Six patients with chronic plaque-type psoriasis were included in the study. They received total body PUVA treatment according to the European PUVA study protocol.[12] During the regular PUVA treatment the test areas selected for the present study were shielded. Three patients were treated concomitantly with etretinate (1·0 mg/kg body weight)[13] because of the severity of their disease. No other topical or systemic treatment was used.

Drug

A liquid preparation of 8-methoxypsoralen (Oxsoralen, Gerot, Vienna, Austria) in soft gelatine capsules was administered orally in a dose of 0·6 mg/kg body weight 1 h before irradiation. The level of this drug preparation in the serum peaks at 1 h.

Irradiation source

A Jobin Yvon HL monochromator (Jobin Yvon, Longjumeau, France) equipped with a Cermax 500 W Xenon lamp was used for phototesting and for subsequent treatments. The half bandwidth was 10 nm. Before each irradiation the irradiance was measured with a thermopile (Müller Elektronik-Optik GmbH, Moosinning, Germany). To avoid stray radiation of shorter wavelengths a 1 mm Schott WG 335 glass filter was used with the 365-nm band. No filter was used for the irradiations with 335 nm, because stray irradiation in the UVB range would not account for more than 1% of the emitted dose.[14] The irradiances were approximately 1·1 mW/cm^2 in the 335-nm region and 3·4 mW/cm^2 in the 365-nm region. Exposure times ranged from 2 to 30 min.

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Phototoxicity testing

The minimal phototoxic dose (MPD) was determined in all six patients by exposing four areas to increasing doses of monochromatic radiation 1 h after ingestion of 8-MOP. Dose increments corresponded to standard MPD testing.[12] MPD testing was performed for both wavelengths, 335 and 365 nm, on the patients' backs. Visual assessment of threshold erythema was done 72 h after exposure.

Treatment protocols

Treatment consisted of four exposures per week. Two 10-cm^2 areas of a psoriatic plaque on the lower back which was covered during the regular total body PUVA treatment received either 335 or 365 nm radiation. A third area remained shielded as an untreated control. The patients were treated according to either protocol 1 or to protocol 2 (see below).

Protocol 1. One MPD of 335 nm radiation was used as the initial dose for both wavelengths. Because the MPD at 335 nm was always lower than that at 365 nm, the 365 nm-treated area always received suberythemogenic doses. Dose increments for both areas were adjusted according to the erythema reaction and/or the response of the psoriatic lesion at 335 nm. Both areas were treated until complete clearance was achieved in one of the treatment sites.

For example, if the 335 nm MPD was 1 J/cm^2 this was also the initial dose for 365 nm; if the dose was increased by 0·5 J/cm^2 in the 335-nm area, the dose in the 365-nm area was increased by the same amount.

Protocol 2. The 335 nm-treated area was initially exposed to the 335 nm MPD and the 365 nm-treated area to the 365 nm MPD. Thus both areas were treated with equally erythemogenic UVA doses. Subsequently, dose increments were based on the response of the respective area. Treatment was continued until complete clearance was achieved in both areas. To avoid a possible bias from varying tissue levels of 8-MOP, the order of irradiation with 335 and 365 nm was alternated.

Results

MPD testing

In all six patients the MPD with 335 nm was approximately 50% lower than with 365 nm (Table 1).

Table 1. Patient data

Patient	Skin type	MPD 335 nm (J/cm^2)	MPD 365 nm (J/cm^2)	Etretinate
W.J.	II	0·5	1·0	Yes
M.L.	II	1·0	2·0	No
P.F.	III	1·0	2·0	Yes
H.L	II	0·2	0·5	No
R.H.	I	0·5	1·0	Yes
B.C.	II	1·0	2·0	No

Table 2. Protocol 1

Patient	Wavelength (nm)	Total cumulative UVA dose (J/cm^2)	Clearance of psoriasis (%)
M.L.	335	20·5	100
	365	20·5	0
P.F	335	9·5	100
	365	9·5	0
W.J.	335	5·9	100
	365	5·9	0

Treatment

Protocol 1. All three patients cleared completely in the 335 nm-treated area but showed no response in the 365-nm area. The cumulative UVA dose required for clearance at 335 nm ranged from 5·9 to 20·5 J/cm^2 and the number of treatments ranged from eight to 18 sessions (Table 2).

Protocol 2. All three patients cleared completely in both the 335 nm- and the 365 nm-treated area, after 8–30 irradiations. The total cumulative doses ranged from 5·0 to 43·4 J/cm^2 and from 10·0 to 83·0 J/cm^2, respectively.

Table 3. Protocol 2

Patient	Wavelength (nm)	Total cumulative UVA dose (J/cm^2) until clearing	Reduction of total UVA dose (%)
B.C.	335	43·4	−44·4
	365	78·0	
H.J.	335	46·0	−44·6
	365	83·0	
R.H.	335	5·0	−50·0
	365	10·0	

The UVA dose required for clearance was 45–50% lower in the 335 nm-treated area as compared to the 365-nm area in all three patients (Table 3).

Discussion

Improvements in photochemotherapy should aim at a reduction of side-effects with increased efficacy. Long-term side-effects in particular such as chronic actinic skin damage and cutaneous carcinogenesis can be assumed to depend on the photobiological properties of the photosensitizing compound and the total UVA dose.[15] The phototoxic reaction of 8-MOP is carcinogenic in animals under certain experimental conditions and there is evidence that some groups of patients have an elevated risk of non-melanoma skin cancer after prolonged PUVA treatment.[15,16] New approaches in photochemotherapy include the development of treatment schedules which permit a reduction in the frequency of treatments and in the total UVA dose. New photoactive compounds with reduced cytogenetic hazards, and their combination with adjuvant therapy, such as the retinoids have been used. A possible improvement may be achieved by the use of wavelengths other than those currently used which peak between 350 and 355 nm.

In our study, MPD testing and the clinical results showed 335 nm to be twice as effective as 365 nm in oral PUVA. This indicates that the antipsoriatic activity and erythemogenicity of 8-MOP may have similar action spectra. The use of other furocoumarins has shown that phototoxicity erythema is not necessarily a requirement for clearing psoriasis.[17] Earlier studies using topical psoralen demonstrated 335 nm to be four times as effective as 365 nm with regard to phototoxic erythema and sunburn cell formation. One possible explanation of this discrepancy is that the systemic use of 8-MOP may result in higher dermal 8-MOP levels, which in combination with the better penetration of 365 nm compensate for the higher absorbance by 8-MOP at 335 nm.

Half of the patients in this study were treated concomitantly with etretinate. However this is unlikely to have affected the results, because the reduction of the UVA dose at 335 nm as compared to 365 nm was always in the same order of magnitude regardless of whether retinoids were added or not.

The question arises whether the higher efficacy of PUVA at 335 nm in clearing psoriasis may contribute to the greater long-term safety of photochemotherapy. The higher yield of tumours in hairless mice found when 8-MOP was used with irradiation between 320 and 335 nm[7] could be interpreted to show that higher efficacy in psoriasis is achieved at the expense of a higher risk of skin tumours. However, by varying treatment schedules and dosimetry it would be possible to determine the least harmful treatment protocol.

References

1 Buck HW, Magnus IA, Porter AD. The action spectrum of 8-MOP for erythema in human skin. Preliminary studies with a monochromator. *Br J Dermatol* 1960; 37: 249–55.

2 Pathak MA. Mechanisms of psoralen photosensitization: *in vivo* biological action spectrum of 8-MOP. *J Invest Dermatol* 1961; 37: 349–407

3 Owens DW, Glickmann JM, Freeman RG, Cranes R. Biological action spectra of 8-MOP determined by monochromatic light. *J Invest Dermatol* 1968; 51: 435–40.

4 Nakayama L, Morikawa F, Fukada M *et al.* Monochromatic radiation and its application—laboratory studies on the mechanism of erythema and pigmentation induced by psoralen. In: *Sunlight and Man* (Fitzpatrick TB, Pathak MA, Harber M, Seiji M, Kukita A, eds). Tokyo: University of Tokyo Press, 1974; 591–611.

5 Cripps DJ, Lowe NJ, Lerner AB. Action spectra of topical psoralens: a re-evaluation. *Br J Dermatol* 1982, 107: 77–82.

6 Young AR, Magnus IA. An action spectrum for 8-MOP induced sunburn cells in mammalian epidermis. *Br J Dermatol* 1981; 104: 541–48.

7 Young AR, Walker SL, Garmyn M. A first approach to an action spectrum for 8-MOP-phototumorigenesis in mice. *J Invest Dermatol* 1988; 90: 175–8.

8 Kaidbey KH. An action spectrum for 8-MOP-sensitized inhibition of DNA synthesis *in vivo*. *J Invest Dermatol* 1985; 85: 98–101.

9 Parrish JA, Fitzpatrick TB, Tanenbaum L, Pathak MA. Photochemotherapy of psoriasis with oral methoxsalen and long wave ultraviolet light. *N Engl J Med* 1974; 291: 1207–11.

10 Wolff K, Fitzpatrick TB, Parrish JA *et al.* Photochemotherapy with orally administered methoxsalen. *Arch Dermatol* 1976; 112: 943–50.

11 Fritsch PO, Gschnait F, Kaaserer G *et al.* PUVA suppresses the proliferative stimulus produced by stripping on hairless mice. *J Invest Dermatol* 1979; 73: 188–90.

12 Henseler T, Christophers E, Honigsmann H *et al.* Skin tumors in the European PUVA study. *J Am Acad Dermatol* 1987; 16: 108–16.

13 Fritsch PO, Hönigsmann H, Jaschke E, Wolff K. Augmentation of oral methoxsalen photochemotherapy with an oral retinoic acid derivative. *J Invest Dermatol* 1978; 70: 178–82.

14 Ortel B, Gange RW. An action spectrum for the elicitation of erythema in skin persistently sensitized by photobound 8-methoxypsoralen. *J Invest Dermatol* 1990; 94: 781–5.

15 Hönigsmann H, Wolff K, Fitzpatrick TB *et al.* Oral photochemotherapy with psoralens and UVA (PUVA): principles and practice. In: *Dermatology in General Medicine* (Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen FK, eds), 3rd edn. New York: McGraw-Hill, 1987; 1533–58.

16 Stern RS, Lange R. Non-melanoma skin cancer occurring in patients treated with PUVA five to ten years after first treatment. *J Invest Dermatol* 1988; 91: 120–4.

17 Honigsmann H, Tanew A, Brücke J, Ortel B. Photosensitizing compounds in the treatment of psoriasis. In: *Photosensitizing Compounds: Their Chemistry, Biology, and Clinical Use* (Ciba Foundation Symposium 146) Chichester: John Wiley & Sons, 1989; 159–70.

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Juvenile spring eruption of the ears: a probable variant of polymorphic light eruption

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Summary We report 18 cases in which a pruritic, erythematous, papular and vesicular eruption developed on the ears following sun exposure. Four of these patients had, on other occasions, suffered from typical polymorphic light eruption. The clinical features, histological changes, and results of phototesting suggest that juvenile spring eruption of the ears is a localized form of polymorphic light eruption.

Juvenile spring eruption of the ears (JSE) is a photodermatosis affecting mainly boys aged 5–12 years, and may occur in epidemics.[1] The eruption, which typically begins in the spring, consists of pruritic erythematous papules affecting the ears, which may progress to vesicles and then crust and heal with little or no scarring. The eruption may give rise to cervical adenopathy.

JSE has received scant attention in the literature but we do not believe that the condition is rare. Although the disease is self-limiting and not, in itself serious, it may cause the patients considerable discomfort and concern. Furthermore, the differential diagnosis includes a spectrum of other photodermatoses.

The aetiology of JSE and its relationship to other photodermatoses have not been fully understood. In the few previous reports histological similarity to erythema multiforme has been emphasized,[2,3] suggesting that JSE may represent a photo-induced variant of this condition.

We report here the clinical features and investigations of a series of 18 cases of JSE.

Case reports

The cases are summarized in Table 1. They came to our attention at three different English dermatological centres in Leicester, London, and Middlesbrough. Cases 1 and 3 were presented at a meeting of the St John's Hospital Dermatological Society and have been previously reported.[4] All but one of our 18 patients were male. All had short hair at the time of the eruption, and most had protuberant ears. All complained of recurrent episodes of redness, itching and blistering over the light-exposed areas of both ears (Fig. 1). The lesions usually appeared in spring and often persisted throughout the summer. Erythema and pruritus typically developed in the evening after exposure to the sun during the day, and, in severe episodes, progressed to blistering over a few hours. Most cases required no treatment, but some reduction in frequency and severity of attacks was obtained by the use of sun screens.

Five cases demonstrating additional features of particular interest are described.

Case 4

A 29-year-old man presented in June complaining of recurrent blistering on the helices of both ears after commencing employment delivering milk early each morning in the spring. He had suffered an identical eruption throughout childhood, until the age of 12, that would appear within an hour of sun exposure and usually progress to blistering.

Examination revealed a maculopapular or erythematous eruption with several vesicles over the helices of both ears. There was slight scarring from previous lesions. The ears were protuberant.

Case 5

An 11-year-old boy with a history of atopic dermatitis presented in May 1989, complaining of recurrent blistering on the ears since the age of 2 that would begin each spring and continue until June. On the evening

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Table 1. Summary of patient data

Case no.	Age at presentation	Age at onset	Age at remission	Skin type	PLE	Family history
1	9	4	Continues	I	No	Brothers Father had JSE in childhood
2	10	9	9	I	No	
3	14	12	14	II	No	None
4	29	1	Continues	II	No	None
5	11	2	Continues	III	Yes	Brothers Father had JSE in childhood
6	13	2	Continues	III	Yes	
7	15	3	11	III	No	
8	16	3	11	III	No	
9	5	3	Continues	I	No	Brothers
10	8	2	Continues	I	Yes	
11	8	2	Continues	—	No	None
12	10	8	Continues	—	No	None
13	11	9	Continues	II	No	Brothers
14	9	7	Continues	II	No	
15	13	10	Continues	II	No	Brothers
16	11	8	Continues	II	No	
17	26	15	Continues	II	No	None
18	58	8	53	I	Yes	Two children and sister developed PLE

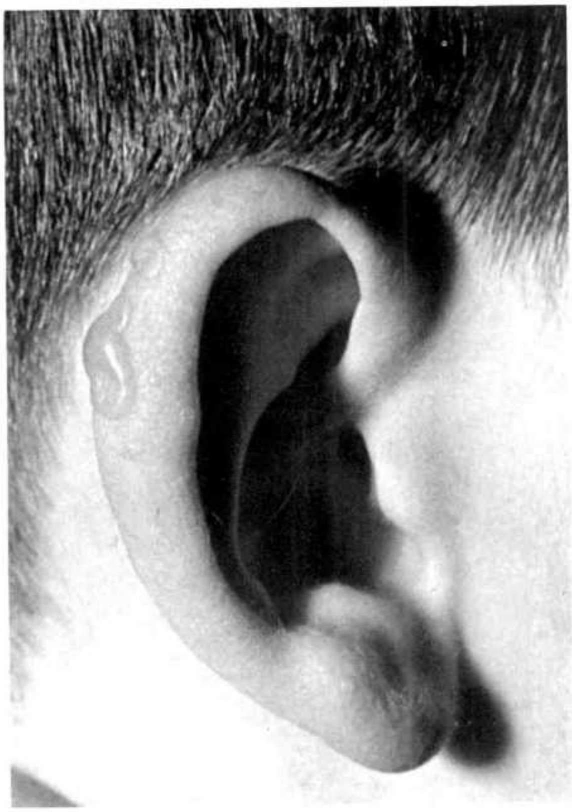

Figure 1. Case 3. Tense bullae on the helix.

following sun exposure he would notice redness and pruritus of the ears. The following day papules and blisters, 4–6 mm in diameter, would develop, scab and heal during the following week leaving minimal scars with either increased or decreased pigmentation. Over the last 2 years he had simultaneously been affected by a more generalized photosensitive, pruritic, erythematous, papular eruption over the face, backs of the hands, and knees, typical of polymorphic light eruption.

This patient had two brothers who had developed a similar eruption affecting only the ears. A third brother is Case 6. His father had developed a similar eruption over the ears as a child.

Case 6

A 13-year-old boy, the brother of Case 5, had developed an eruption each year from the age of 2. Initially this involved the ears, face, knees and hands, but over the 2 years prior to presentation only the ears and face had been affected. The lesions on the ears were pruritic erythematous papules and blisters and elsewhere, there were pruritic erythematous papules. There was a history of hay fever.

Case 10

An 8-year-old boy presented in April 1989 with a pruritic erythematous, papular and vesicular eruption over the helices of both ears. This had developed each spring from the age of two and persisted throughout the summer. In June 1989, during a sunny period, he developed for the first time a maculopapular erythematous and pruritic rash over both thighs and upper limbs which resolved without treatment after 48 h. A biopsy from the helix of an ear 18 h after the eruption began is described below.

His brother had a similar eruption over the ears on one occasion at the age of 5.

Case 18

A 58-year-old man of fair complexion gave a history of a red, pruritic blistering eruption on his ears from 8 years of age. This would occur mainly during summer holidays in coastal or mountainous regions and occurred intermittently until the age of 53. The eruption would settle spontaneously within 2 weeks of returning home. In addition, during a holiday in Yugoslavia at the age of 43, he first developed a pruritic erythematous maculopapular eruption over the limbs and other sun-exposed areas. This continued to develop following exposure of the limbs to the sun during holidays, and resolved within 5 days of returning home. Symptomatic improvement was obtained from topical antihistamines, topical steroid preparations and chloroquine. A similar widespread eruption had affected two daughters and a sister.

Porphyrin levels and autoantibodies (Cases 1, 3–8, 11, 12)

Porphyrin levels in blood, urine and stool, and antinuclear antibodies, including anti-double-stranded DNA, anti-SSA and anti-SSB titres were normal.

Histology: Cases 4 and 10

Case 4. There was a dense, predominantly perivascular and periadnexal lymphohistiocytic infiltrate throughout the dermis (Fig. 2). In places subepidermal spongiotic vesicles were seen. Immunofluorescent staining revealed no abnormal deposition of immunoglobulin, fibrin or complement. Staining for surface markers on the dermal infiltrate showed this to be predominantly lymphocytic and composed of approximately equal numbers of CD4$^+$ and CD8$^+$ cells.

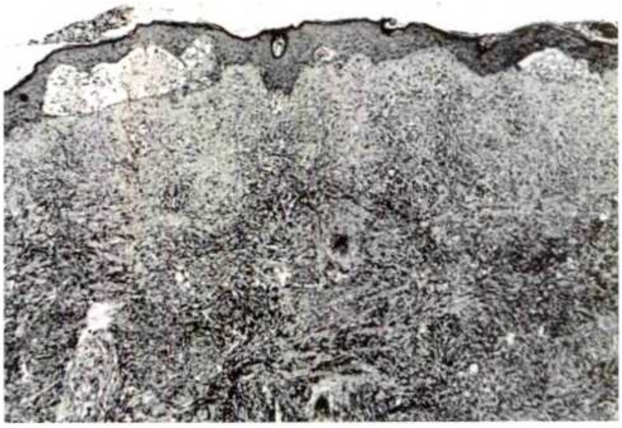

Figure 2. Case 4. A dense infiltrate is seen throughout the dermis. (Haematoxylin and eosin × 12).

Case 10. A mild perivascular mononuclear cell infiltrate and mild spongiosis were present, but there was no other abnormality noted.

Light testing: Cases 3–8

Erythemal responses of the skin of the upper back to a series of increasing doses of narrow wavebands of ultraviolet and visible light at 300, 307·5, 320, 340, 360, 380, 400 and 500 nm from an irradiation monochromator (model 498/01; Applied Photophysics, London, U.K.) were normal except for mildly increased sensitivity at 340 nm (minimal erythema dose $\leqslant$ 10,000 mJ/cm^2; normal $\geqslant$ 15,000) and 360 nm (MED $\leqslant$ 12,500 mJ/cm^2; normal $\geqslant$ 15,000) in Case 4. In two patients, reactions of uninvolved skin of the back to a single exposure to a series of doses of up to two minimal erythema doses of broad band UVA (Mutzhas UVASUN 2000) and UVB (Philips TL 20W/12) irradiation were normal. Attempts to reproduce the reaction on the upper part of the ears of these two patients by a single exposure to between half and one minimal erythema dose of solar simulated irradiation (Kratos 2500 W, xenon arc filtered, LH 152N/3SS) were unsuccessful.

Discussion

Juvenile spring eruption of the ears has received little attention since Anderson *et al.*[1] described an outbreak in a children's summer camp in Surrey, where 121 of 150 children at the camp in early April 1953 were affected. In all but one case, in which there were lesions on a hand, the eruption involved only the ears. Although the camp was closed due to alarm over possible infectious aetiology, this was not confirmed and the authors concluded that the eruption may have been due to a combination of sunlight and cold air. It was more frequent and more severe in boys than in the girls whose hair covered their ears. The affected children were outdoors for most of the day in sunny but cold weather with a moderate wind. These authors were also aware of sporadic cases seen by dermatologists and general practitioners.

Burckhardt[2] in 1942 described a very similar condition in 18 patients (14 boys, one girl, three adults) in Switzerland. One patient had suffered from recurrent episodes for 25 years. The histology of one case somewhat resembled either erythema multiforme or lupus erythematosus but may be interpreted from the description as compatible with polymorphic light eruption. Burckhardt also studied a school class containing 50 boys and 50 girls during a period of unusually fine spring weather; 44 of the boys developed papules and crusting of the ears, whereas only one girl with swept back hair developed the changes. Burckhardt considered this to be an actinic reaction, the girls being protected by their long hair. More recently, Baran and Civatte reported 10 cases of JSE.[3] These authors reported histological findings in two cases and supported Burckhardt's view that the features were similar to those of erythema multiforme.

Prior to the presentation of Patient 1, JSE had not been diagnosed by a Leicester dermatologist for at least 10 years; however, the dermatosis may be more common than has been previously believed. The self-limiting nature of the condition may account for the infrequency of dermatological referrals.

The clinical picture in JSE is usually characteristic and in typical cases patients can be reassured that the disease is benign. However, other conditions may give rise to a vesicular or bullous eruption on the ear and, if only this site is affected, be clinically indistinguishable from JSE.

Erythema multiforme may affect the ear and may also rarely be light-induced. No histological resemblance of JSE to erythema multiforme has been found in our cases. Hydroa vacciniforme is a severe, but rare photodermatosis affecting children. Although this may produce lesions on the ears these do not generally occur in isolation, the cheeks and nose also usually being affected. This disease produces severe scarring, not a feature of JSE. Furthermore, the histology of our case is not that of hydroa vacciniforme. Actinic prurigo also predominantly affects children, giving rise to pruritic excoriated papules over light-exposed skin. However, hands, as well as nose and cheeks, are usually affected and vesicles are unusual. The porphyrias may induce photosensitivity and blistering of the skin but not confined to the ears. In our patients, excess porphyrins were not detected.

JSE therefore appears quite distinct from the conditions described above. However, the onset of pruritic papules or vesicles several hours after sun exposure is characteristic of the common photodermatosis polymorphic light eruption (PLE)[5] and JSE has other similarities to this disease.

The positive family history, obtained in a large proportion of our cases of JSE, is not infrequent in PLE.[6,7] Although PLE is more common in adults, the condition is by no means rare in children,[5,8] as demonstrated by Cases 5, 6 and 10. Like JSE, PLE tends to develop in discrete episodes, resolve spontaneously without scarring, and recur for years, and is not confined to patients with any particular skin complexion.[9] Classical PLE often has a wider body distribution than JSE, but not uncommonly appears at only one site.[5] Although blistering of the skin is rare in PLE, this may be more common on the ear. The development of typical PLE in four of our patients who at other times suffered from typical JSE, further suggests that the two conditions are related. Normal responses to monochromatic irradiation would be consistent with PLE.[10] Reproduction of the eruption with solar-simulated irradiation might be more successful with repeated exposure. The histology[10,11] and immunohistology[12] in our cases is entirely compatible with that of polymorphic light eruption. We therefore conclude that JSE may be regarded as clinical variant of PLE.

References

1 Anderson D, Wallace HJ, Howes EIB. Juvenile spring eruption *Lancet* 1954; i: 755–6.

2 Burckhardt W. Über eine im Fruhling, besonders an den Ohren, auftretende Lichtdermatose. *Dermatologica* 1942; 86: 85–91.

3 Baran R, Civatte J La photodermatose printanière juvénile. *Ann Dermatol Venereol* 1980; 107: 537–41.

4 Berth-Jones J, Norris PG, Graham-Brown RAC, Burns DA. Juvenile spring eruption of the ears. *Clin Exp Dermatol* 1989; 14: 462–3.

5 Jansen CT. The natural history of polymorphous light eruption. *Arch Dermatol* 1979; 115: 165–9.

6 Ros AM, Wennersten G. Current aspects of polymorphous light eruptions in Sweden. *Photodermatology* 1986; 3: 298–302.

7 Jansen CT. Heredity of chronic polymorphous light eruptions. *Arch Dermatol* 1978; 114: 188–90.

8 Frain-Bell W, Dickson A, Herd J, Sturrock I. The action spectrum in polymorphic light eruption. *Br J Dermatol* 1973; 89: 243–9.

9 Epstein JH Polymorphous light eruption. *J Am Acad Dermatol* 1980; 3: 329–43.

10 Frain-Bell W. *Cutaneous Photobiology*. Oxford: Oxford University Press, 1985; 30–32

11 Hood AF, Elpern DJ, Morison WL. Histopathologic findings in papulovesicular light eruption. *J Cutan Pathol* 1985; 13: 13–21.

12 Norris PG, Morris J, McGibbon DM *et al.* Polymorphic light eruption: an immunohistochemical study of evolving lesions. *Br J Dermatol* 1989; 120: 173–84.

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Polymorphic light eruption with contact and photocontact allergy

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Summary We report a patient with long-standing polymorphic light eruption (PLE) who developed a photocontact allergy to mexenone and several contact allergies. The occurrence of multiple contact and photocontact allergies in PLE and the possible relationship of such allergies to the pathogenesis of PLE are discussed.

Polymorphic light eruption (PLE) is a recurrent, persistent, idiopathic, light-sensitive, inflammatory dermatosis.[1–3] The lesions occur on light-exposed areas of the body hours to days after sun exposure and can take many different forms, but in an individual patient one type of eruption usually predominates, the small papular variety being the commonest. Lesions resolve spontaneously within days if further sun-exposure is avoided. PLE occurs in spring or early autumn in most patients. Phototests to determine the action spectrum of PLE implicate the long-wave ultraviolet UVA band in 56% of cases, UVB in only 17%, and both UVA and UVB in 26%.[4]

PLE is the commonest photodermatosis, with an estimated overall prevalence of 10–20%.[5,6] In one study,[7] it occurred in 38% of patients investigated for suspected photodermatoses, and of the patients with PLE almost 7% had a positive photopatch test and about 10% had positive patch tests. In another study,[8] about 12·5% had positive photopatch tests and 8% had positive patch tests.

We report a patient with disabling PLE complicated by the development of multiple contact allergies and a photocontact allergy. This case report illustrates the importance of carrying out patch and photopatch testing in PLE to eliminate aggravating factors, and the possible relevance of allergens in the development of PLE and chronic actinic dermatitis is discussed.

Case report

A 68-year-old woman presented to the Department of Dermatology in 1982. She gave a 16-year history of itchy red papules and plaques on the face and papules on the dorsa of the hands occurring within 1–8 h after sun exposure. The eruption cleared in 2 days if further sun exposure was avoided. She had had psoriasis since the age of 30. UVB therapy for psoriasis, when she was in her forties, had no adverse effect. Methotrexate had been commenced at the age of 60, several years after the onset of photosensitivity, with good control of the psoriasis. She also gave a history of developing a generalized itching and blotchy erythema in warm environments. She originally worked as an orthopaedic nurse, but for the last 10 years had worked in an administrative role. On examination there were discrete, erythematous papules and plaques on the face and papules on the dorsa of both hands, consistent with PLE, and minimal psoriasis on the thighs.

A skin biopsy from a lesion showed a perivascular infiltrate of lymphocytes, histiocytes and eosinophils in the superficial and mid-dermis, with prominent upper dermal oedema and mild epidermal spongiosis, consistent with PLE. Patch testing to the European Standard Series and photopatch testing was negative. Other investigations, including screening tests for porphyrins, anti-Ro, anti-La and antinuclear antibodies were normal. The most likely diagnosis was thought to be PLE and she was advised to minimize her sunlight exposure and to use sunscreens.

Although sunscreens were initially protective, some appeared to cause a rash, and the patient also noted adverse reactions to elastoplast and perfumes. Further patch testing in 1989 at the Mater Hospital revealed nine positive reactions, considered to be relevant (Table 1). The histology of a further skin lesion was again consistent with PLE. She was referred to St John's Hospital, where patch testing confirmed sensitivity to

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Table 1. Positive patch and photopatch tests

	Suspected allergen contacts
Positive patch tests	
Fragrances	
Perfume mix (+3)	
Balsam of Peru (+2)	Perfumes
Oak moss absolute (+3)	Perfumes, cosmetics
Usnic acid (+2)	Some antibiotic creams and perfumes
Atranorin (+3)	Perfumes
Preservatives	
Imidazolidinyl urea (+1)	Cosmetics
Diazolidinyl urea (+1)	Cosmetics
Formalin (+2)	(Many products)
Miscellaneous	
Colophony (Rosin) (+2)	Adhesive tape, elastoplast
Epoxy resin (+2)	Adhesives
Coniferyl benzoate (+2)	
Positive photopatch test	
Benzophenone-10 (+2)	Uvistat sunscreen

the same substances, but in addition allergies to imidazolidinyl urea and diazolidinyl urea, and photopatch testing revealed a positive photocontact reaction to benzophenone-10 (Table 1). Photo-provocation testing with a solar simulator reproduced characteristic lesions of PLE, and monochromator irradiation testing indicated marked photosensitivity to UV radiation from 300 to 340 nm. These investigations were indicative of severe PLE, complicated by several contact allergies and a photocontact allergy to benzophenone-10. While desensitization with PUVA was initially considered, avoidance of relevant allergens has resulted in a marked improvement of the patient's condition.

Discussion

The diagnosis of PLE depends on the temporal relationship of the rash to sun exposure and complete resolution of lesions between attacks, supported by clinical and histological findings and phototests, and the exclusion of lupus erythematosus. Biopsy of lesions is not, by itself, diagnostic,[8] but diagnosis is facilitated by the reproduction of lesions using photoprovocation by solar-simulated radiation. Assessment of a patient's spectral sensitivity by monochromator irradiation tests is only occasionally useful, as not all patients react to narrow wavebands.

This patient lies at the severe end of the spectrum of PLE,[5] with marked UVB and UVA photosensitivity. However, multiple positive patch tests are somewhat unusual in PLE. Patients with chronic actinic dermatitis (CAD),[9] also termed 'persistent light reaction', or photosensitivity dermatitis with actinic reticuloid syndrome, by comparison are more likely than patients with PLE to have multiple allergies,[7,8] which may include Compositae,[10] lichens,[8] and fragrances.[11] Some patients with PLE therefore, like ours, give a picture more in keeping with overlapping PLE and CAD, or possibly progression from PLE to CAD. Patients with features of both disorders would not be unexpected as both disorders may be a consequence of a similar pathogenetic pathway involving delayed-type hypersensitivity.[12,13] Exposure of the skin to UV-radiation and phototoxins, either exogenous such as lichen acids and Compositae,[14] or endogenous, may lead to the formation of an as yet ill-defined neoantigen to which a delayed cell-mediated hypersensitivity reaction develops. This neoantigen may be induced in each episode of relevant UV-exposure. Induction of endothelial leucocyte adhesion molecule-1 (ELAM-1) and intercellular adhesion molecule-1 (ICAM-1) occurs in similar patterns in lesions of both PLE[15] and delayed-type hypersensitivity,[16] but in a different pattern in UVB-induced erythema,[16] and may determine the type of inflammation that occurs.

Oak moss, a perfume fixative, is a common cause of contact allergy to perfumes, especially in men.[17] Oak moss extract is made from lichens, especially *Evernia prunastri* (oak moss) and *Pseudevernia furfuracea* (tree moss). Some of the contact allergens in lichens are known and include atranorin, evernic acid, usnic acid and fumarprotocetraric acid.[18,19] Lichen compounds appear to be strongly allergenic,[20] causing mainly allergic contact dermatitis.[18,19,21] *In-vitro* testing suggests that lichens are also phototoxic compounds capable of causing cell membrane damage.[22] In one study of photosensitive patients tested with various lichen compounds, atranorin was observed to be the most frequently involved allergen and multiple concomitant allergies to lichen acids were also observed.[20] Usnic acid has antimicrobial properties and is also used in some topical antibiotic preparations, therefore exposure in a nurse is not unexpected. Contact allergy to lichen substances may be acquired not only through contact with perfumed topical preparations containing oak moss,[17] but also through direct contact with the plants in nature.[17,18]

Colophony, oak moss and balsam of Peru, to which this patient reacted, may contain similar compounds.[17] It may be difficult to tell whether reactions to colophony and balsam of Peru are concomitant reactions or cross reactions. Imidazolidinyl urea and diazolidinyl urea are preservatives used in cosmetics. They may cause allergic contact dermatitis, and may cross-react with one another.[23] They also release formaldehyde, but the significance of this to formaldehyde-sensitive individuals is uncertain.[23]

In addition to allergic contact dermatitis, the photosensitivity in this patient was complicated by the development of photoallergic contact dermatitis to benzophenone-10 (2-hydroxy-4-methoxy-4-methylbenzophenone, mexenone). Benzophenones are widely used in sunscreens, with benzophenone-3 (oxybenzone) being the most common.[24] They may cause both type I and type IV hypersensitivity.[25] Both allergic[7,26–28] and photoallergic contact dermatitis[7,24,29,30] to benzophenones occur often in patients with pre-existing light-sensitive disorders. Benzophenone-10 has rarely been reported to cause sensitivity, but can produce both allergic and photoallergic contact dermatitis.[31,32] The incidence of allergic reactions to benzophenones may be underestimated because patch and photopatch testing to benzophenones is not done often enough. Sunscreens play a major role in the prophylaxis of chronic photosensitive disorders. They are also paradoxically responsible for a significant number of contact and photocontact allergic reactions in photosensitive patients.[7,8] As sunscreen-induced photodermatitis can closely simulate photodermatoses, its occurrence may not be recognized. If a patient with photosensitivity fails to benefit from a sunscreen or their condition deteriorates while using it, then the components of the sunscreen should be checked by patch and photopatch testing.

Patients with PLE and CAD appear to be at increased risk of acquiring contact and/or photocontact allergies to chemicals in topical preparations. Apart from the risk involved in the frequent use of sunscreens and the application of topical preparations to irritated and eczematous skin,[33] there may be an underlying susceptibility to the development of type IV hypersensitivity. Our patient illustrates the importance of patch testing and adequate photopatch testing in the photodermatoses,[34] and also the close relationship of PLE and chronic actinic dermatitis.

Acknowledgments

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References

1 Hölzle E, Plewig G, von Kries R, Lehmann P. Polymorphous light eruption. *J Invest Dermatol* 1987, 88: 32s–38s.

2 Epstein JH. Polymorphous light eruption. *J Am Acad Dermatol* 1980; 3: 329–43.

3 Jansén CT, Karvonen J. Polymorphous light eruption: a seven year follow-up evaluation of 114 patients *Arch Dermatol* 1984, 120: 862–5.

4 Ortel B, Tanew A, Wolff K, Honigsmann H. Polymorphous light eruption: action spectrum and photoprotection. *J Am Acad Dermatol* 1986; 14: 748–53.

5 Morison WL, Stern RS. Polymorphous light eruption: a common reaction uncommonly recognised *Acta Derm Venereol (Stockh)* 1982; 62: 237–40.

6 Ros A, Wennersten G. Current aspects of polymorphous light eruption in Sweden. *Photodermatol* 1987; 3: 298–302.

7 Thune P, McFadden N, Jansén C *et al.* The Scandinavian multicenter photopatch study 1980–1985. In: *Current Topics in Contact Dermatitis*. Berlin, Heidelberg: Springer-Verlag, 1989; 487–92.

8 Thune P, Eeg-Larsen T. Contact and photocontact allergy in persistent light reactivity. *Contact Dermatitis* 1984; 11: 98–107.

9 Hawk JLM, Magnus IA. Chronic actinic dermatitis—an idiopathic photosensitivity syndrome including actinic reticuloid and photosensitive eczema. *Br J Dermatol* 1979; 101 (Suppl. 17): 24.

10 Frain-Bell W, Johnson BE. Contact allergic sensitivity to plants and the photosensitivity dermatitis and actinic reticuloid syndrome. *Br J Dermatol* 1979; 101: 503–12.

11 Addo HA, Ferguson J, Johnson BE, Frain-Bell W. The relationship between exposure to fragrance materials and persistent light reaction in the photosensitivity dermatitis and actinic reticuloid syndrome. *Br J Dermatol* 1982; 107: 261–74.

12 Ralfkiaer E, Wantzin GL, Stein H, Mason DY. Photosensitive dermatitis with actinic reticuloid syndrome; an immunohistochemical study of the cutaneous infiltrate. *Br J Dermatol* 1986; 114: 47–56.

13 Norris PG, Morris J, McGibbon DM *et al.* Polymorphic light eruption: an immunopathological study of evolving lesions. *Br J Dermatol* 1989; 120: 173–83.

14 Murphy GM, White IR, Hawk JLM. Allergic airborne contact dermatitis to Compositae with photosensitivity—chronic actinic dermatitis in evolution. *Photodermatol* 1990; 7: 38–40.

15 Norris PG, Barker JNWN, MacDonald DM *et al.* Expression of endothelial leukocyte adhesion molecule-1 (ELAM-1) and intercellular adhesion molecule-1 (ICAM-1) in induced lesions of polymorphic light eruption. (Abstract). Photodermatology Group, British Association of Dermatologists Annual Meeting, Cambridge, England, 1989.

16 Norris P, Thomas S, Thornhill M *et al.* Expression of adhesion molecules ELAM-1 and ICAM-1 parallels leukocyte recruitment in cutaneous inflammation: A comparison of ultraviolet-B erythema and delayed type hypersensitivity. *Clinical Dermatology in the Year 2000*, London, May 1990, Programme and Book of Abstracts

17 Goncalo F, Cabral F, Goncalo M. Contact sensitivity to oak moss. *Contact Dermatitis* 1988; 19: 355–7.

18 Dahlquist I, Fregert S. Contact allergy to atranorin in lichens and perfumes. *Contact Dermatitis* 1980; 6: 111–19

19 Dahlquist I, Fregert S. Atranorin and oak moss contact allergy *Contact Dermatitis* 1981; 7: 168–9.

20 Thune P, Solberg YJ. Photosensitivity and allergy to aromatic lichen acids. *Contact Dermatitis* 1980; 6: 64–71.

21 Thune P, Solberg Y, McFadden N *et al.* Perfume allergy due to oak moss and other lichens. *Contact Dermatitis* 1982; 8: 155–8.

22 Wennersten G. Photodynamic reactions induced by compounds derived from lichens. *Acta Derm Venereol (Stockh)* 1979; **59:** 197–200.

23 Fisher AA. Formaldehyde. In: *Contact Dermatitis.* (Fisher AA, ed), 3rd edn. Philadelphia: Lea and Febiger, 1986; 570–81

24 Knobler E, Almeida L, Ruzkowski AM *et al.* Photoallergy to benzophenone. *Arch Dermatol* 1989; **125:** 801–4.

25 Ramsay DL, Cohen HJ, Baer RL Allergic reaction to benzophenone; simultaneous occurrence of urticarial and contact sensitivities. *Arch Dermatol* 1972; **105:** 906–8.

26 Thompson G, Maibach H, Epstein J Allergic contact dermatitis from sunscreen preparations complicating photodermatitis. *Arch Dermatol* 1977; **113:** 1252–3.

27 Baer RL, Ramsay DL. Polyvalent light sensitivity (persistent light reactivity?): allergic contact sensitivity to Sulisobenzone. *Arch Dermatol* 1971; **104:** 446–8.

28 Pariser RS. Contact dermatitis to dioxybenzone. *Contact Dermatitis* 1977; **3:** 172.

29 Thune P. Contact and photocontact allergy to sunscreens. *Photodermatol* 1984; **1:** 5–9.

30 Holzle E, Plewig G. Photoallergische Kontaktdermatitis durch benzophenonhaltige Sonnenschutzpratrarate. *Hautarzt* 1982; **33:** 391–3.

31 Millard LG, Barrett PL. Contact allergy from mexenone masquerading as an exacerbation of light sensitivity. *Contact Dermatitis* 1980; **6:** 222–3.

32 Burry JN. Photoallergies from benzophenones and beta-carotene in sunscreens. *Contact Dermatitis* 1980; **6:** 211–12.

33 Larsen WG. Perfume dermatitis. In: *Contact Dermatitis.* (Fisher AA, ed), 3rd edn. Philadelpia: Lea and Febiger, 1986; 394–404.

34 Jansén CT, Wennersten G, Rystedt I *et al.* The Scandinavian standard photopatch test procedure *Contact Dermatitis* 1982; **8:** 155–8.

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Recurrent porokeratosis

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Summary A 63-year-old man developed linear, annular and accuminate lesions which showed the histological features of porokeratosis. The lesions cleared spontaneously, recurred several months later and ulcerated, then cleared again. The spontaneous resolution and recurrence of the lesions is a hitherto undescribed pattern of porokeratosis.

The clinical presentations of porokeratosis may vary. To date five patterns of porokeratosis are recognized: porokeratosis of Mibelli, disseminated superficial actinic porokeratosis (DSAP), linear porokeratosis, porokeratosis punctata discreta and porokeratosis palmaris et plantaris disseminata.[1] The porokeratotic lesions are fixed and refractory to most treatments.[2] Destructive therapy such as cryotherapy may flatten the lesions, but porokeratotic lesions rarely clear. We report a patient who developed recurrent linear, annular and accuminate, keratotic lesions of porokeratosis.

Case report

In 1986 a 63-year-old married man presented to the department of genito-urinary medicine with intensely pruritic, painful lesions on the shaft of the penis. There was no associated penile discharge although he experienced severe pain during sexual intercourse. He was otherwise well. After an infectious aetiology had been ruled out, he was referred to the dermatology department.

Examination showed well demarcated, slightly hypopigmented scaly plaques which resembled psoriasis, on the shaft of the penis and prepuce. The patient was treated with a mild topical steroid preparation. Six weeks later he developed similar annular, scaly lesions on the scrotum. The lesions were 0·5–1·5 cm in diameter, hypopigmented in the centre and hyperpigmented around the edges and resembled lichen planus. At the same time symmetrical, linear, hyperpigmented, keratotic lesions developed in the flexures of the arms and the upper part of the thighs. The sun-exposed areas were spared. Detailed examination showed darkly pigmented linear lesions with a slightly raised border and surrounding erythema in the flexures of the arms.

Smaller perifollicular papules extended down the forearms and up the upper arm. Along the outer margins

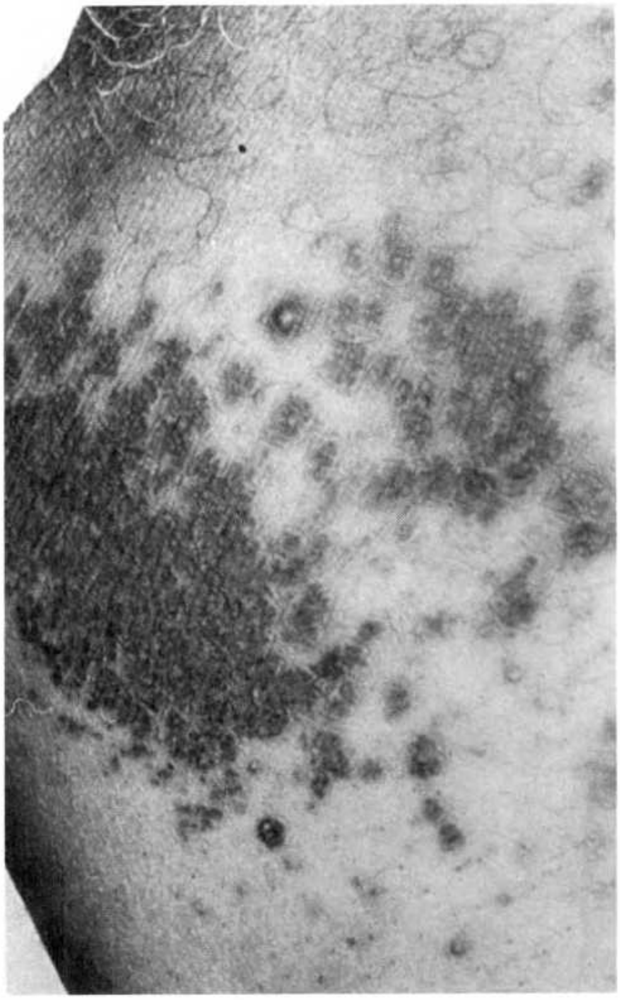

Figure 1. Annular porokeratotic lesions on the thigh

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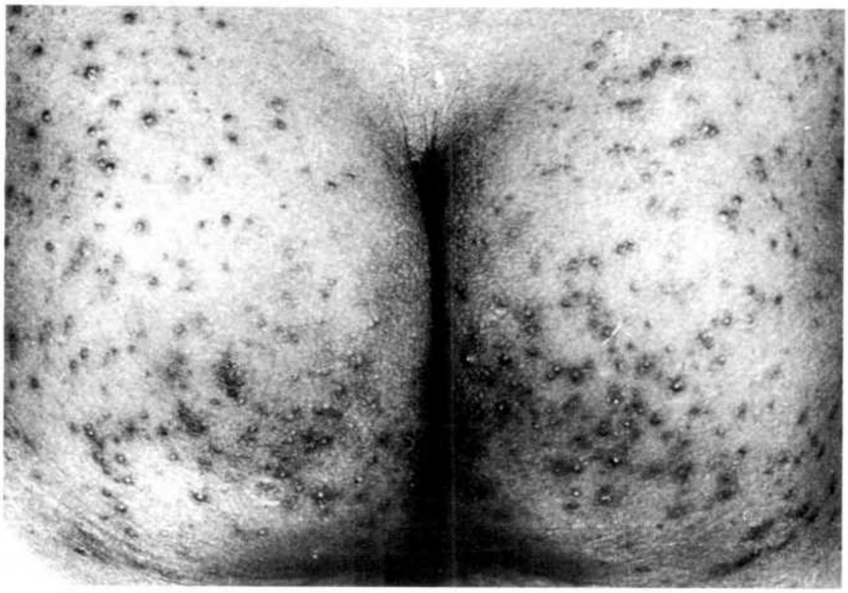

Figure 2. Accuminate porokeratotic lesions on the buttocks.

of the plaques on the thighs were smaller annular lesions with a keratotic rim (Fig. 1). A few weeks later minute, keratotic 'spines' which resembled keratosis pilaris developed on the buttocks, lower back and upper arms (Fig. 2). Some of the spines reached 3 mm in length, showed a follicular distribution and developed on an erythematous base which later became pigmented. There were no mouth lesions and the palms, soles and nails were normal.

There was no family history of a similar eruption. Previously he had transient parasthesiae down the left arm, but all neurological investigations were normal.

Investigations

Full blood count and biochemical investigations were normal. VDRL was positive to a titre of 8, indicative of previous treponemal disease. The patient was from Jamaica and had yaws as a child. HTLV-1 antibody was negative and protein electrophoresis was normal. The ESR was 10 mm/h.

Histopathology

Biopsies from the linear lesion on the arm, the annular lesion on the upper thigh and a spiny lesion on the

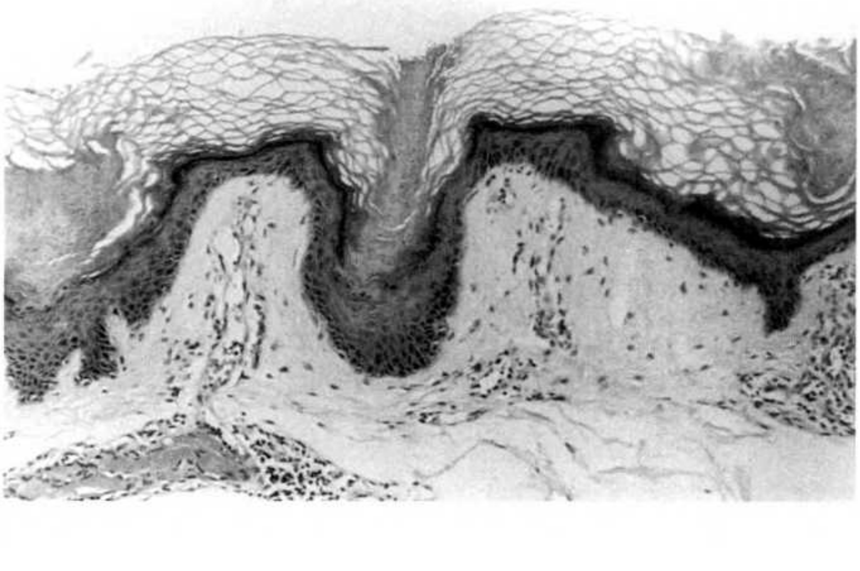

Figure 3. Histology of lesion from the thigh showing a cornoid lamella consisting of a column of parakeratotic cells arising from the epidermis. (haematoxylin and eosin, × 125).

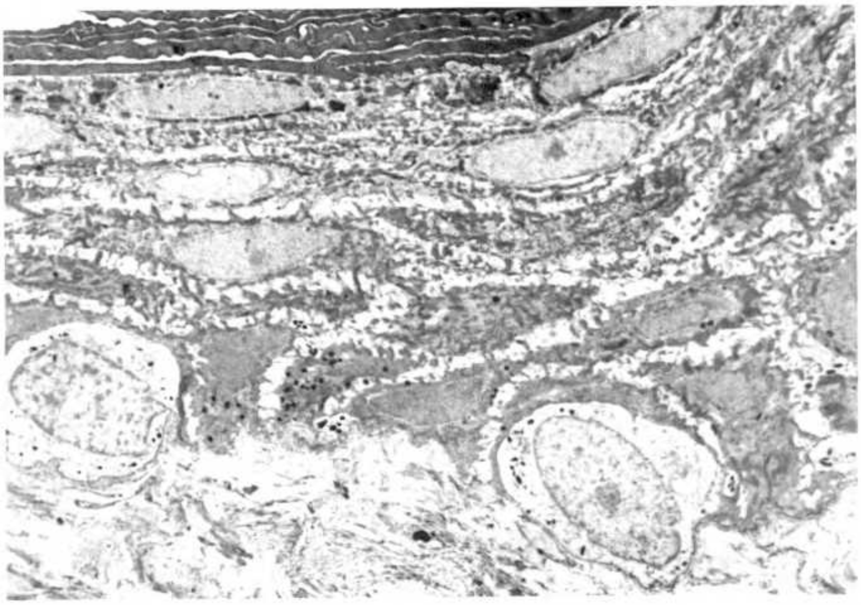

Figure 4. Electron micrograph of epidermis underneath the cornoid lamella. Condensation of the tonofilaments can be seen in the periphery of some of the keratinocytes. (× 2500).

buttock all showed similar histological features. The epidermis was hyperkeratotic with basket-weave orthokeratosis. In between the normal keratin there were columns of parakeratotic cells forming cornoid lamellae (Fig. 3). The rest of the epidermis showed no cytological atypia or basal cell hyperplasia. There was a dense inflammatory infiltrate scattered throughout the dermis, not particularly related to adnexal structures or hair follicles. Lymphocytes and monocytes were the predominant cells. The blood vessels in the upper dermis in some sections showed thrombotic occlusion and there was red blood cell extravasation.

Electron microscopy

Studies were performed on tissue that was retrieved from the paraffin blocks and reprocessed. This ensured that the tissue selected for electron microscopy corresponded to the areas of the parakeratotic columns as seen on light microscopy. Ultrastructurally, the keratinocytes beneath the parakeratotic columns showed condensation of tonofilaments at their periphery (Fig. 4) as has been previously described,[3] but no pyknotic nuclei or large perinuclear vacuoles; features interpreted as being degenerative. Sato *et al.*[4] have described dyskeratotic cells composed of nuclear remnants and aggregate tonofilaments but these were not seen here.

Course of the disease

Topical steroids or salicylic acid produced no clinical improvement. Six months after presentation, the lesions on the arms and legs developed superficial ulcers and became secondarily infected. The latter settled with systemic antibiotics and the porokeratotic lesions returned to their initial appearance. After a further 3 months, the lesions cleared spontaneously from the thighs and arms leaving post-inflammatory hyperpigmentation. The accuminate lesions persisted. Two months later, the porokeratotic lesions on the arms and thighs recurred and again resisted various topical therapies. After a further 4 months the lesions again cleared spontaneously and to date (over 12 months later) have not returned.

Discussion

The hallmark of porokeratosis is the cornoid lamella[5] and although not diagnostic it is always found in the different forms. However, cornoid lamellae may occur with several other conditions.[5] In our patient, the initial clinical lesions were suggestive of psoriasis or lichen planus but when the annular lesions developed with a firm keratotic rim, the diagnosis of porokeratosis became more obvious. The linear narrow lesions on the arms were most unusual.

The keratotic spines have been described previously in porokeratotic punctate keratoderma[1] of the palms and soles. In our patient, the palms and soles were spared throughout the course of the disease. A family was described with palmar and annular lesions over the whole body.[6] The latter resembled those seen in our patient and showed the typical histological features of porokeratosis. However in that report there was a strong family history of porokeratosis and almost all of those patients had palmar or plantar involvement. They also differed from our patient as their eruptions persisted.

Our patient's eruption was unusual as there were annular, linear and accuminate lesions which all showed the histological features of porokeratosis. Also, there was spontaneous ulceration and subsequent clearance. Ulceration has been described previously[7] but complete spontaneous clearing has not. Also the ulceration was followed by atrophy, whereas our patient's skin shows no atrophy at present. Although the lesions recurred at the same site, the patient remains in remission 1 year later. A further interesting observation was the presence of thrombotic lesions in the dermis. No obvious cause could be found to explain this feature.

There is no family history of a similar disease and the disease spontaneously resolved leaving only post-inflammatory hyperpigmentation. This was clearly not porokeratosis of Mibelli, DSAP, punctate porokeratosis or linear porokeratosis although there were linear lesions present. We wish to record this case as another clinical pattern of porokeratosis.

References

1 Friedman SJ, Herman PS, Pittelkow MR, Su WPD. Punctate porokeratotic keratoderma. *Arch Dermatol* 1988; **124**: 1678–82.

2 Wolff-Schreiner EC. Porokeratosis. In: *Dermatology in General Medicine* (Fitzpatrick TB, Eisen AZ, Wolff K *et al*., eds), 3rd edn. New York. McGraw-Hill International Book Co, 1987; 534–40.

3 Mann PR, Cort DF, Fairburn EA, Abdel-Aziz A. Ultrastructural studies on two cases of porokeratosis of Mibelli. *Br J Dermatol* 1974, **90**: 607–17.

4 Sato A, Anton-Lamprecht I, Schnyder UW. Ultrastructure of inborn errors of keratinization. VII. Porokeratosis Mibelli and disseminated superficial actinic porokeratosis. *Arch Dermatol Res* 1976; **255**: 271–84.

5 Wade TR, Ackerman AB. Cornoid lamellation: a histological reaction pattern. *Am J Dermatopath* 1980, **2**: 5–14.

6 Guss SB, Osbourn RA, Lutzner, MA. Porokeratosis plantaris, palmaris, et disseminata. *Arch Dermatol* 1971; **104**: 366–73.

7 Razack EMA, Natarajan M. Ulcerative systematized porokeratosis (Mibelli). *Arch Dermatol* 1977; **113**: 1583–4.

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Acral pseudolymphomatous angiokeratoma of children (APACHE): a case report and immunohistological study

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Summary Acral pseudolymphomatous angiokeratoma of children (APACHE) is a new clinical entity that is characterized by angiomatous papules on the extremities. We report a case APACHE in a 14-year-old Japanese girl with asymptomatic red and violaceous papules and nodules on the ring finger of the left hand.

Histological and immunohistochemical studies of our patient indicated that APACHE is a pseudolymphoma rather than an angiokeratoma.

Cutaneous pseudolymphoma represents a benign reactive hyperplasia of lymphoid elements in response to various stimuli. Recently, Ramsay *et al.*[1] reported a variant of this condition that occurred on the extremities of children as acral pseudolymphomatous angiokeratoma of children (APACHE). We report a case of this condition in a Japanese girl.

Case report

A 14-year-old Japanese girl presented with red–violaceous and firm papules on the dorsum of the ring finger of the left hand that were asymptomatic and which had developed over the previous 5 years. They had gradually enlarged and new papules developed despite treatment with topical steroids.

On examination there were linearly arranged scaly red papules and nodules varying in size from 3 to 7 mm and with a tendency to coalesce on the dorsum of the finger (Fig. 1a). The medial part of the nail showed partial onycholysis and deformity (Fig. 1b). There were no other lesions. Laboratory investigations that included complete blood count and liver function tests were all normal, as was the urinalysis.

On histology there was a dense cellular infiltrate just beneath the flattened epidermis (Fig. 2a) and the cells consisted of lymphocytes, histiocytes (Fig. 2c) and there were many plasma cells (Fig. 2d). There were occasional multinucleate giant cells (Fig. 2c). There were several lymphocytes seen in the overlying epidermis that appeared flattened and showed hydropic degeneration of the basal cell layer (Fig. 2b). There was mild hypergranulosis and focal parakeratosis (Figs 2a, b). Immunohistochemical studies showed that there were Leu 3a positive (helper/inducer) lymphocytes in the upper part of the infiltrate, whereas in contrast there were Leu 2a positive (cytotoxic/suppressor) lymphocytes in the deeper part of the infiltrate. Almost all the epidermotropic cells were Leu 3a positive. Small numbers of Leu 16 positive B cells were to be found focally distributed in the infiltrate.

Intralesional injections of triamcinolone 0·5 mg every 2 weeks produced a gradual diminution in the size of the lesions which eventually disappeared. However, some of the lesions have recurred in the past 2 months.

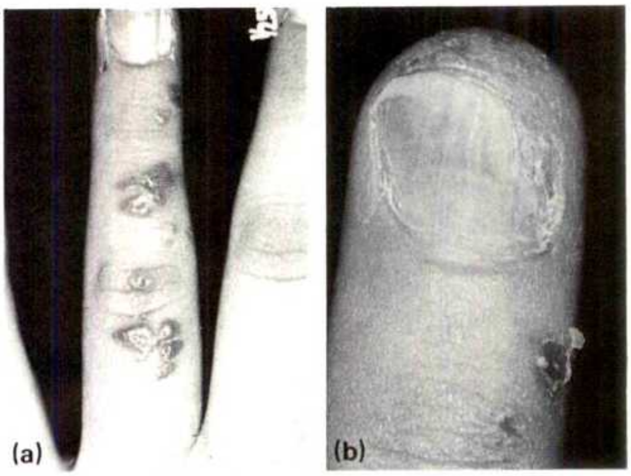

Figure 1. (a) Linear papules on the dorsum of the left fourth finger. (b) Partial onycholysis and deformity of the fingernail.

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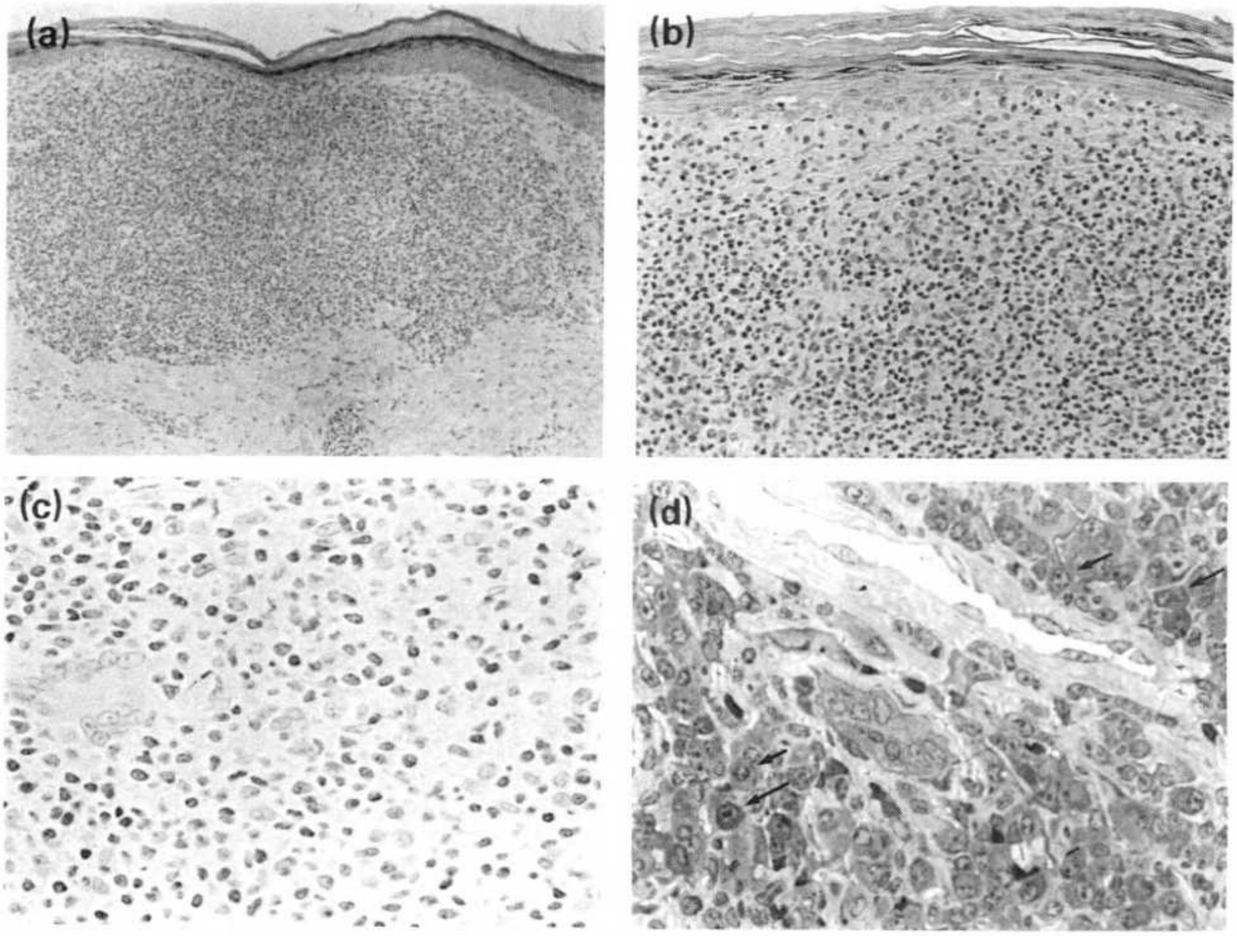

Figure 2. (a) Circumscribed infiltrate beneath a flattened epidermis (× 30). (b) Hyperkeratosis, partial hypergranulosis and hydropic degeneration of epidermis with epidermotropism of lymphocytes (× 75). (c) Lymphohistiocytic infiltrate with small numbers of Langerhans and giant cells (× 150). (d) Semithin Epon section showing plasma cells (arrows) stained with toluidine blue (× 150).

Discussion

Our case was unusual in that there was a linear arrangement of the red–violaceous papules and nodules on one finger. However, the presence of a striking focal upper dermal lymphohistiocytic infiltrate strongly suggested the diagnosis of pseudolymphoma. The finger is an unusual location for pseudolymphoma, which usually affects the face and head.[2] In 1988 Ramsay *et al.*[1] proposed a new entity of acral pseudolymphomatous angiokeratoma in children (APACHE). In our case there were multiple lesions in a linear fashion on one finger with involvement of the nail as seen in lichen planus. The histology of the dense cellular infiltrate in the dermis corresponded to that of a pseudolymphoma, but there was a lack of prominent thickened capillaries[1] which suggests that they are not a consistent feature of this disorder. There were epidermal changes in our case that consisted of liquefaction degeneration of the basal cells with the presence of Leu 3a lymphocytes. These features were not previously reported in the cases of APACHE or pseudolymphoma. Leu 3a positive T cells appear to have a strong affinity for the epidermis and it has been suggested that they can induce secondarily the lichenoid tissue reaction.[3] This lichenoid infiltrate, suggestive of lichen planus or lichen nitidus, was noted in our case though the clinical features were not of these disorders.

References

1 Ramsay B, Dahl MGC, Malcolm AJ *et al.* Acral pseudolymphomatous angiokeratoderma of children (APACHE). *Br J Dermatol* 1988; **119** (Suppl. 33): 13.

2 Tomita T, Masu S, Noguchi T. Three cases of lymphadenosis benigna cutis. *Jap J Clin Derm* 1988; **32**: 133–7.

3 Shiohara T, Moriya N, Nagashima M. The lichenoid tissue reaction: a new concept of the pathogenesis. *Int J Dermatol* 1988; **27**: 365–74.

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Etretinate and AIDS-related Reiter's disease

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Summary We report a case of a 34-year-old homosexual man who developed Reiter's disease with severe cutaneous and joint involvement shortly after he was diagnosed as having acquired immunodeficiency syndrome (AIDS). Therapy with potent topical steroids and oral methotrexate was ineffective, but etretinate at a dose of 0·75 mg/kg resulted in rapid clearing of the skin lesions and a marked improvement of his arthralgia.

It is now well established that the sudden onset of psoriasis/Reiter's disease can herald the development of AIDS.[1,2] Such cases can be difficult to treat,[1,2] with an appreciable risk of further immunosuppression from most systemic therapies. Our case illustrates the progression of what at first appeared to be psoriasis into Reiter's disease and we review the associated therapeutic problems.

Case report

A 34-year-old Caucasian homosexual man presented with an acute paronychia affecting his right ring finger. This began at about the same time as he developed AIDS, which was diagnosed on the basis of an episode of *Salmonella* dysentery complicated by septicaemia, ulcerative oesophageal candidiasis, chronic perianal herpes simplex infection, a positive antibody to HIV-1 and a low absolute CD4 lymphocyte count of 40/μl (CD4/CD8 ratio of 0·2). He was HIV-1 antibody positive for 18 months prior to presentation at the skin clinic and was taking zidovudine 250 mg six times daily, amoxycillin 250 mg twice daily for chronic salmonellosis, acyclovir 200 mg t.d.s. and ketoconazole 200 mg once daily. The patient recalled having three episodes of non-specific urethritis in the past, all of which occurred over 5 years prior to the development of AIDS. There was no family history of psoriasis or joint problems.

Other digits and toes quickly became involved, and a well-demarcated glazed erythema with overlying yellow scale developed on the perionychial skin. Fusiform swellings of the terminal interphalangeal joints were noted (Fig. 1) which were associated with pain and reduced movement of the affected joints. A Köbner reaction in a tattoo was noted that initially affected only the red pigment areas. Similar but larger well-demarcated, scaly dull-red plaques appeared on the trunk and limbs and a diagnosis of psoriasis was made.

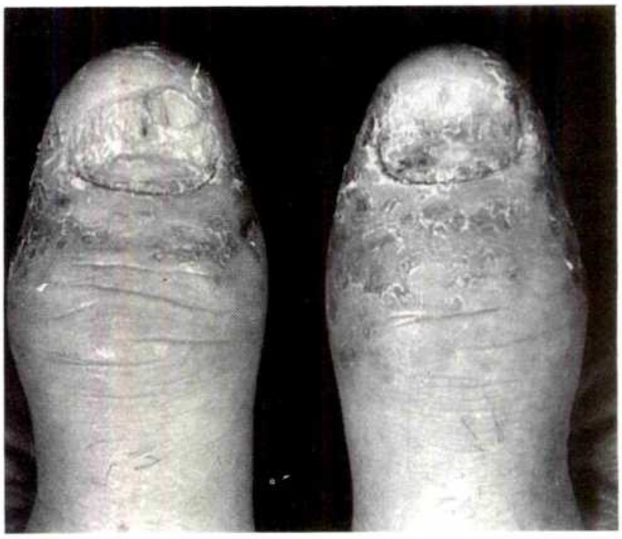

Figure 1. Gross swelling of terminal interphalangeal joints of both thumbs with associated nail dystrophy and perionychial erythema and scaling.

Two months later, his joints became worse with continuous pain in the distal parts of the fingers and feet and treatment with non-steroidal anti-inflammatory agents were unhelpful. He developed diffuse pain in the right mid-foot, and low back pain associated with stiffness. Both great toes became very tender and swollen and prevented the patient from walking. The nail plates were replaced by heaped-up yellow scale which eventually shed. Discrete, scaly red macules developed on the palms (Fig. 2) and similar lesions with overlying conical yellow scales on the soles and sides of the feet with the

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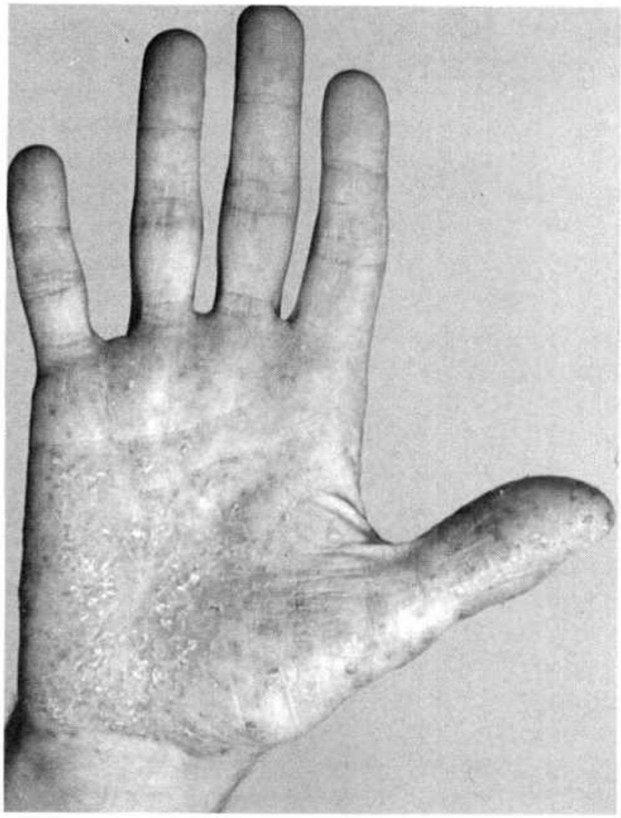

Figure 2. Discrete dull red scaly macules on palms.

clinical appearance of keratoderma blenorrhagica. New, less well-demarcated lesions appeared in the scalp which were oozing and crusted. An acceleration of his male pattern alopecia was also noted. The flexures were affected by an oozy erythematous eruption consisting of discrete circinate erosions 3–5 mm in diameter with a periphery of scale, which eventually became confluent (Fig. 3). A circinate balanitis was noted, and small erosions appeared on the buccal mucosa and soft palate. There was no conjunctivitis and he had no symptoms and signs suggestive for a urethritis or prostatitis. Cultures taken for *Chlamydia* were negative. A diagnosis of Reiter's disease was made on the basis of a bacillary dysentery, the pattern of arthritis, and the characteristic mucocutaneous lesions.[3]

Relevant investigations at this stage showed a haemoglobin count of 10·3 g/dl, a total white cell count of $1{\cdot}6 \times 10^9$/l with a CD4 count of 20 μl and CD8 count of 165 μl. HIV core antibody was positive and HIV antigen was negative. Liver function tests and renal function were normal, and rheumatoid factor, antinuclear factor and syphilis serology were all negative. X-rays of the hands and feet showed soft tissue swelling, osteopenia, small erosions and periostitis of the affected distal interphalangeal joints. Skin swabs taken from oozing areas on the toes, scalp and both arms, and skin biopsies of the right forearm and the medial border of the left foot were sent for culture, but failed to grow any bacteria, atypical mycobacteria or fungi apart from an isolate of *Aspergillus fumigatus* which was considered to be a contaminant. The histology of the skin lesions was compatible with Reiter's disease with a thick parakeratotic stratum corneum overlying an acanthotic epidermis and an upper dermal infiltrate of predominantly mononuclear cells. The epidermis was oedematous and numerous vacuolated keratinocytes were seen which in parts formed cysts. One skin biopsy taken from the arm showed lobular vascular dilatations filled with blood and prominent endothelial cells close to the hyperplastic epidermis. Interstitial separation of collagen bundles by spindle cells associated with haemorrhage and inflammatory cells including neutrophils and plasma cells were seen. Warthin–Starry stain did not reveal any bacilli.

Topical treatment of the cutaneous lesions with clobetasol propionate ointment was unhelpful, despite

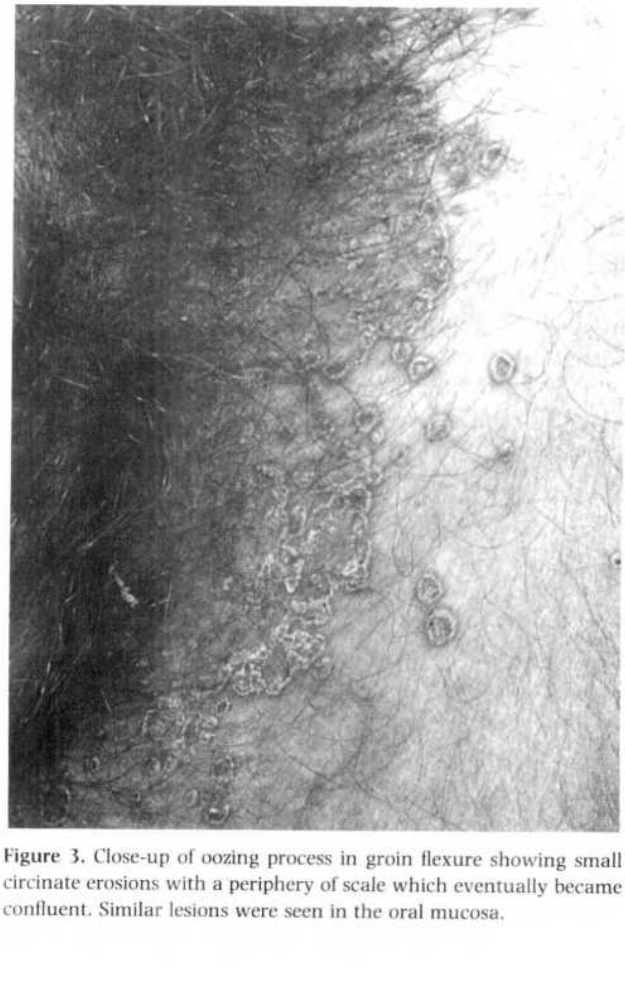

Figure 3. Close-up of oozing process in groin flexure showing small circinate erosions with a periphery of scale which eventually became confluent. Similar lesions were seen in the oral mucosa.

polythene occlusion to the feet. Potassium permanganate soaks (1:8000 dilution) produced some improvement as regards drying the oozing areas in the groins and feet. In view of the severity of the cutaneous involvement, systemic PUVA therapy was started only to be abandoned after two treatments because the patient had difficulty in standing. Oral methotrexate at a dose of up to 30 mg once a week was tried but after 6 weeks of treatment there was no benefit and the already low white cell count began to fall further. All drugs including methotrexate and zidovudine were then stopped.

In view of the vascular lesions that were seen in one of the biopsy specimens, and on the basis of a report that had just appeared at that time on the use of oral erythromycin for the treatment of AIDS-related angiomatosis,[4] erythromycin 250 mg q.d.s. was tried. This resulted in a considerable improvement in the degree of oozing and erythema and pain in the feet, but the beneficial effects lasted for only 2 weeks, the cutaneous process reverting to its original state despite continuing erythromycin therapy.

Etretinate was then given, initially at a dose of 25 mg once daily (0·5 mg/kg), with some improvement after 10 days in the degree of erythema and scaling of the feet. The low back pain was worse but this was probably due to the Reiter's disease rather than the etretinate. The dose of etretinate was increased to 40 mg once daily (approximately 0·75 mg/kg) resulting in clearance of all the skin lesions and a marked improvement of the arthralgia and the back pain. The swelling of the terminal interphalangeal joints was markedly reduced and the patient was again able to walk.

The patient remained free from cutaneous lesions for a further 3 months when he was admitted in a confused state. Cutaneous lesions of ecthyma gangrenosum were noted on the extremities and cultures of the exudates from these lesions revealed *Pseudomonas* spp. which was also found on blood culture. Despite treatment with intravenous antibiotics, the patient died 10 days later.

Discussion

Psoriasis and Reiter's disease probably form part of a clinical spectrum of disease expression in genetically predisposed individuals.[3,5] The distinction between the two diseases is especially blurred in AIDS,[1] as was seen in our patient. The classical triad of urethritis, conjunctivitis and arthritis described by Reiter[6] is probably seen in only a third of cases.[7] Our patient fulfils the accepted criteria[8,9] for diagnosing Reiter's disease in having a history of bacillary dystentery, an asymmetrical peripheral arthritis of greater than 1 month's duration with predominantly lower limb involvement, and characteristic mucocutaneous lesions which included keratoderma blenorrhagica, circinate flexural erosions, balanitis circinata and oral involvement.

A number of reports[1,2,10–12] have described the onset and exacerbation of psoriasis and Reiter's disease in AIDS. In such patients, several forms of the disease may co-exist and psoriasis may progress to Reiter's disease, allowing investigation of immunopathological mechanisms common to both diseases.[13] It is suggested that the onset of psoriasis/Reiter's disease in an HIV-positive patient is associated with a poor prognosis.[10] Another feature common to these patients is the refractory nature of the disease to conventional therapy. Various systemic therapies have been suggested but all share the potential problem of further immunosuppression in an already profoundly immunocompromised individual.

Should topical therapy fail, zidovudine seems a logical therapeutic approach in such patients (C. Farthing, personal communication), and early results appear encouraging.[14,15] The antipsoriatic effect of zidovudine appears to be dose-dependent and related to the development of erythrocyte macrocytosis rather than helper T-cell function, which has worsened in some patients despite a sustained remission of their skin disease.[14] Our patient had already been taking zidovudine for 6 months at a dose of 250 mg six times daily and this was probably not helpful, as reducing or stopping his zidovudine on three occasions because of his precipitously low white cell count did not appear to be associated with an exacerbation of his Reiter's disease.

PUVA appears to be an ideal treatment for this problem, but may be impractical because of the patients' debility. Also as HIV particles have been found in the Langerhans cells of psoriatic scales[16] there may be a risk of transmission to other patients having PUVA-treatment. Experience of treated AIDS-related prurigo with ultraviolet light suggests that the risk of ultraviolet light-induced Kaposi's sarcoma[10,17] may have been overestimated, but this is still an area of concern.

Methotrexate at a dose of 30 mg once weekly was ineffective in the treatment of our patient and possibly contributed to a profound neutropenia that necessitated stopping all his drugs. Since then there have been similar reports of disappointing results with methotrexate in this subgroup of patients, and this drug has been shown to possibly accelerate the course of AIDS in three patients[1,18] and to have caused profound neutropenia in three others,[2,10] HIV encephalopathy in one case and fulminent Kaposi's sarcoma in another.[10]

There was a temporary response of our patient's skin disease to oral erythromycin. The lobular vascular dilatation seen in one of our patient's skin biopsies was similar to that seen in epithelioid angiomatosis,[19] a condition which responds to erythromycin therapy. However, bacilli were not seen in the lesions of our patients.

Oral etretinate has been shown to be of benefit in AIDS-related psoriasis[10] and resulted in complete clearance in our patient of the cutaneous lesions and a marked improvement in his arthralgia. The etretinate was well tolerated with no obvious side-effects. A similar success was reported by Belz *et al.*[13] in another patient with AIDS-related Reiter's disease who responded to a daily dose of 1 mg/kg of etretinate and suggested that the beneficial effect was produced by restoring Langerhans cell populations in psoriatic lesions.

This is the first report from the U.K. to suggest that etretinate is an extremely effective and safe treatment for the cutaneous and joint manifestations of AIDS-related Reiter's disease.

Acknowledgments

We wish to thank Dr Malcolm Gellan and Dr Tom McManus for allowing us to publish this case.

Addendum

We have recently seen another Caucasian 42-year-old homosexual patient with plaque psoriasis of over 20 years' duration, who suddenly developed extensive and unstable psoriasis. Suspecting HIV-1 infection, antibody testing was performed with his consent and which was positive. This patient also developed an oligoarthropathy, circinate balanitis, oral lesions, and as well a conjunctivitis. There was a history of at least eight episodes of non-specific urethritis over the last 15 years. We have treated this patient so far with zidovudine 200 mg every 4 h, and at the present time his skin is virtually clear and his joint symptoms have improved. However, should he relapse then he will be treated with etretinate. This patient with HIV infection also illustrates the development of Reiter's disease from typical psoriasis.

References

1 Winchester R, Bernstein DH, Fischer HD *et al.* The co-occurrence of Reiter's syndrome and acquired immunodeficiency. *Ann Intern Med* 1987; 106: 19–26.

2 Johnson TM, Duvic M, Rapini RP, Rios A. AIDS exacerbates psoriasis. *N Engl J Med* 1985; **313**: 1415.

3 Baker H. Reiter's disease. In: *Textbook of Dermatology* (Rook A., Wilkinson DS, Ebling JG, Champion RH, Burton JL, eds). Oxford: Blackwell Scientific Publications, 1986; 1533–42.

4 Axiotis CA, Schwartz R, Jennings TA, Glaser N. AIDS-related angiomatosis. *Am J Dermatopath* 1989; **11**: 177–81.

5 Ingram GJ, Scher RK. Reiter's syndrome with nail involvement; is it psoriasis? *Cutis* 1985; **36**: 37–40.

6 Reiter H. Uber eine bisher unerkannte Spirochaeteninfektion (Spirochaetosis arthritica). *Deutsch Med Wochenschr* 1916; **42**: 1535–36.

7 Arnett FC, McClusky OE, Schacter BZ, Lordon RE. Incomplete Reiter's syndrome; discriminating features of HLA-W27 in diagnosis. *Ann Intern Med* 1976; **84**: 8–12.

8 Calin A, Fox R, Gerber RC, Gibson DJ. Prognosis and natural history of Reiter's syndrome. *Ann Rheum Dis* 1979; 38(Suppl. 1): 29–31.

9 Fisk P. Reiter's disease. *Br Med J* 1982; **284**: 3–4.

10 Duvic M, Johnson TM, Rapini RP *et al.* Acquired immunodeficiency syndrome—associated psoriasis and Reiter's syndrome. *Arch Dermatol* 1987; 123: 1622–32.

11 Lazar AP, Roenigk HH. AIDS and psoriasis. *Cutis* 1987; **39**: 347–51.

12 Sadick NS, McNutt NS, Kaplan MH. Papulosquamous dermatoses of AIDS. *J Am Acad Dermatol* 1990; **22**: 1270–7.

13 Belz J, Breneman DL, Nordland JJ, Solinger A. Successful treatment of a patient with Reiter's syndrome and acquired immunodeficiency syndrome using etretinate. *J Am Acad Dermatol* 1989; **20**: 898–903.

14 Kaplan MH, Sadick NS, Wieder J *et al.* Antipsoriatic effects of zidovudine in human immunodeficiency virus—associated psoriasis. *J Am Acad Dermatol* 1989; **20**: 76–82.

15 Diez F, del Hoyo M, Serrano S. Zidovudine treatment of psoriasis associated with acquired immunodeficiency syndrome. *J Am Acad Dermatol* 1990; **22**: 146–7.

16 Tschachler E, Groh V, Popovic M *et al.* Epidermal Langerhans cells: a target for HTLV-III/LAV infection. *J Invest Dermatol* 1987, **88**: 233–7.

17 Stingl G, Gazze-Stingl LA, Aberer W, Wolff K. Antigen presentation by murine epidermal Langerhans cells and its alteration by ultraviolet B light. *J Immunol* 1981, **127**: 1707–13.

18 Lambert RE, Kaye BR. Methotrexate and the acquired immunodeficiency syndrome. *Ann Intern Med* 1987; **106**: 773.

19 Berger TG, Tappero JW, Kaymen A, Le Boit PE. Bacillary (epithelioid) angiomatosis and concurrent Kaposi's sarcoma in acquired immunodeficiency syndrome. *Arch Dermatol* 1989; **125**: 1543–7.

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Correspondence

Bruising and susceptibility to solar urticaria

Sir, The clinical diagnosis of solar urticaria generally relies on a history of wealing beginning within 5–10 min after exposure to ultraviolet or visible radiation and persisting for 1–2 h. We wish to draw attention to a characteristic clinical feature of this condition, to our knowledge previously unreported, which can prove helpful in diagnosis.

A 53-year-old Caucasian male presented with a 15-month history of wealing of non-covered sites beginning approximately 15 min following exposure to strong sunlight. He reported exaggerated susceptibility to wealing of any bruised skin areas which either developed weals in isolation following exposure to mild or moderately intense sunlight or reacted more rapidly and intensely than surrounding skin in strong sunlight. No abnormal urticarial responses were demonstrated on phototesting skin of the upper back with narrow wavebands of 300–600 nm light from an irradiation monochromator. However, repeat testing 24 h later on a mildly bruised venesection site in the left antecubital fossa, using identical irradiation doses, demonstrated immediate wealing at 400–500 nm.

This phenomenon of increased sensitivity of bruised skin has been confirmed by several other solar urticaria patients on direct questioning. There is now good evidence[1,2] that in solar urticaria there is a precursor substance present in the serum, which on absorption of light energy becomes antigenic such that it can bind to IgE-bearing mast cells, causing degranulation. Thus, an increased local concentration of this serum factor would be expected to render bruised skin especially sensitive.

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References

1 Horio T, Minami K. Solar urticaria: photoallergen in a patient's serum. *Arch Dermatol* 1977; 113: 157–60.
2 Leenutaphong V, Holzle E, Plewig G. Pathogenesis and classification of solar urticaria: a new concept. *J Am Acad Dermatol* 1989; 21: 237–40.

ADONIS 000709639100093C

Isotretinoin and wax epilation

Sir, Cutaneous side-effects as a result of treatment with isotretinoin are common. Frequent adverse reactions are cheilitis, dry skin, dry nose and mouth, pruritus and increased skin fragility. We report a case of erosions that occurred on the sites of wax epilation on the face of a woman who was on treatment with oral isotretinoin.

A 30-year-old woman who was otherwise healthy was referred to our clinic with a 12-year history of severe cystic acne. She was prescribed isotretinoin at a dosage of 0·5 mg/kg/day and by the twelfth week of therapy there was a considerable improvement with the disappearance of the papules and nodules and a marked reduction in the size and number of the other lesions. Previously she had had many wax depilations of her face without any problem which were carried out for idiopathic hirsutism. However, after 14 weeks of therapy with isotretinoin she had a wax epilation of the skin on the upper lip and chin and this resulted in oedema with confluent erosions over the entire area. The wounds healed without scarring and there was no pigmentary change.

Retinoid drugs have a profound effect on many different aspects of skin biology.[1] Increased skin fragility is well described but the pathogenesis is at present unclear. In our patient, epidermal damage may have been due to an increased skin fragility that has resulted from treatment with isotretinoin. I suggest that patients who are on this retinoin should be advised against having epilation, paricularly on their face.

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1 Elias PM. Epidermal effects of retinoids: supramolecular observations and clinical implications. *J Am Acad Dermatol* 1986, 15: 797–809.

ADONIS 000709639100094P

'Tin-tack' sign in seborrhoeic dermatitis

Sir, The 'tin-tack' sign, the appearance of spiny processes that project from the undersurface of detached scale and correspond to follicular openings, has been reported in discoid lupus erythematosus,[1] pemphigus foliaceus[2] and drug-induced lichen planus.[3] We now report the sign in seborrhoeic dermatitis.

A 69-year-old man presented with a 2-year history of a troublesome scaly eruption on the face. His medical problems included chronic schizophrenia and temporal arteritis for which he was taking thioridazine 50 mg t.d.s. and prednisolone 40 mg daily. On examination his appearance was unkempt and he had Parkinson's disease. There was a macular erythematous rash with overlying thick yellow greasy scale, predominantly affecting the central area of the face and most marked on the nose, with discrete areas of macular erythema and heaped-up scale scattered on the forehead and temples. Removal of the scale revealed underlying erythema and on the undersurface of the detached scale there were spiky projections corresponding to dilated follicular openings (Fig. 1). Spiny processes without overlying scale also projected from follicular openings in adjacent areas. These were examined with Parker Ink in KOH (50% of each) and revealed numerous corneocytes

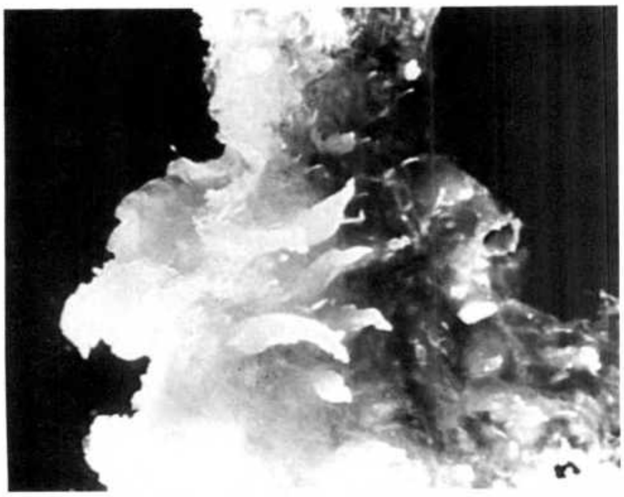

Figure 1. Light microscopic view (× 20) of detached scale showing follicular projections.

and *Pityrosporum* spores of the oval type. Histological examination showed areas of spongiosis with overlying focal parakeratosis and mild dermal inflammatory infiltrate without acantholysis. A PAS stain showed many pityrosporal yeasts of the oval type in the stratum corneum. Direct skin immunofluorescence was negative. The diagnosis of seborrhoeic dermatitis due to *Pityrosporum ovale* was made and the patient was treated with twice daily 2% ketoconazole cream and more frequent washing. Four weeks later the facial rash had completely resolved.

We attribute the severity of seborrhoeic dermatitis in our patient to an increased pool of sebum from his Parkinson's disease maintained by infrequent washing.[4] Seborrhoeic dermatitis can be confused with pemphigus foliaceus[5] which was considered in our patient because of the 'tin-tack' sign. It was excluded by histology and immunfluorescence, and the presence of many oval yeasts and the rapid response to antipityrosporal therapy confirmed our initial clinical diagnosis of seborrhoeic dermatitis. Although the 'tin-tack' sign is not mentioned in recent descriptions of seborrhoeic dermatitis, its occurrence was recognized as long as 70 years ago.[6] The findings in our patient and others[1–3] demonstrates that the 'tin-tack' sign is not disease-specific.

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References

1 Perry HO, Brunsting LA. Pemphigus foliaceus. *Arch Dermatol* 1965; **91:** 10–23.
2 Paramsothy Y, Lawrence CM. 'Tin-tack' sign in localised pemphigus foliaceus. *Br J Dermatol* 1987; **116:** 127–9.
3 Cox NH, Tapson JS, Farr PM. Lichen planus associated with captopril: a further disorder demonstrating the 'tin-tack' sign. *Br J Dermatol* 1989; **120:** 319–21.
4 Cowley NC, Farr PM, Shuster S. The permissive effect of sebum in seborrhoeic dermatitis: an explanation of the rash in neurological disorders. *Br J Dermatol* 1990; **122:** 71–76.
5 Burton JL, Rook A, Wilkinson DS. Seborrhoeic dermatitis. In: *Textbook of Dermatology* (Rook AJ, Wilkinson DS, Ebling FJG, Champion RH, Burton JL, eds), 4th edn, Vol. 1. Oxford: Blackwell Scientific Publications, 1986.
6 Stelwagon, Henry W. *Dermatitis Seborrhoeic: A Treatise on Diseases of the Skin*, 8th edn. Philadelphia and London: W.B. Saunders Company, 1918: 341–54.

ADONIS 0007096391000954

Azathioprine in the treatment of *Parthenium* dermatitis

SIR, Airborne contact dermatitis due to the plant *Parthenium hysterophorus* is the cause of considerable morbidity in India. These patients are difficult to manage. We report two cases of persistent dermatitis due to *Parthenium* that were successfully treated with oral azathioprine.

A 42-year-old male engineer (Case 1) with a 4-year history of airborne contact dermatitis due to *P. hysterophorus* was admitted to hospital with a severe exacerbation of his dermatitis and was treated with 30 mg prednisolone daily. The dose of the steroids was reduced and he was then given oral psoralen photochemotherapy with trimethylpsoralen. As his response to this treatment was not satisfactory, he was also given azathioprine in doses of 100 mg daily. After 1 month he had improved and was discharged from hospital to continue on azathioprine and oral psoralen photochemotherapy with sun exposure. After 2 weeks this was discontinued but he remained on azathioprine and when examined 3 months after discharge from hospital he was virtually free of all lesions.

The second case was a 55-year-old female teacher with airborne contact dermatitis due to *P. hysterophorus* who had relapsed after discontinuing oral hyposensitization treatment because of epigastric pain. She was treated with 30 mg prednisolone and 100 mg azathioprine daily. Over the next few weeks the dose of steroids was reduced and after 3 months of therapy she was completely free of all lesions.

P. hysterophorus belongs to the family of Compositae and this plant is a common cause of airborne contact dermatitis in India with features suggestive of a photosensitivity.[1] Oral hyposensitization has been reported by us to be effective in one patient,[2] but in two others it was unhelpful. Although our first case was on PUVA treatment as well as on azathioprine we believe that the latter was responsible for his improvement. Azathioprine has been reported to be effective in chronic actinic dermatitis[1,4] and in longstanding photoeczematous dermatitis.[5] We consider that the immunosuppressive drug azathioprine is effective in the management of cases of airborne contact dermatitis due to *Parthenium* that are not responding to other more conventional treatments.

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References

1 Bhutani LK, Rao DS. Photocontact dermatitis caused by *Parthenium hysterophorus*. *Dermatologica* 1978; 157: 206–9.
2 Srinivas CR, Krupashankar DS, Singh KK *et al.* Oral hyposensitization in *Parthenium* dermatitis. *Contact Dermatitis* 1988; 18: 242–3.
3 Leigh IM, Hawk JLM. Treatment of chronic actinic dermatitis with azathioprine. *Br J Dermatol* 1984; 110: 691–5.
4 Murphy GH, White JR, Hawk JLM. Allergic airborne contact dermatitis to Compositae with photosensitivity—chronic actinic dermatitis in evaluation. *Photodermatol Photoimmunol Photomed* 1990; 7: 38–9.
5 Epstein JH. Polymorphic light eruption. *Dermatologic Clinics* 1986; 4: 243–51.

ADONIS 000709639100096H

Etretinate therapy and thrombocytopenia

Sir, Etretinate is effective in the treatment of severe psoriasis, especially generalized pustular psoriasis of the Von Zumbush type and psoriatic erythroderma. As with other retinoids, there are a number of adverse reactions to etretinate but even in high doses, the drug is thought to be non-toxic to the bone marrow and haematological reactions are rare.[1]

We wish to report a 54-year-old male who was admitted to our hospital in December 1988 with fever and generalized pustular psoriasis who developed thrombocytopenia following treatment with etretinate. He had previously had a 2-month history of plaque psoriasis. Laboratory investigations showed acute-phase reactants and a slight increase of γ-glutamyl transferase, and the platelet count was only marginally reduced, being 132×10^9/l.

He was initially treated with systemic steroids with improvement and subsequently etretinate was given up to 60 mg daily and the steroids discontinued. Etretinate was later reduced to 25 mg daily, but because of a recurrence was increased to 35 mg per day and in March 1989 to 50 mg per day. The platelet count at that time was 146×10^9/l. In April 1989 there was a relapse of his pustular psoriasis and he was readmitted to hospital. In addition to having etretinate 50 mg per day he was given systemic steroids. Laboratory investigations showed a hypertriglyceridaemia and a thrombocytopenia of 36×10^9/l that was confirmed by repeated tests. Investigations as to the cause of his thrombocytopenia were normal and a bone marrow aspirate showed erythroid and myeloid hyperplasia and numerous megakaryocytes. It was decided to stop treatment with etretinate and to give oral psoralen photochemotherapy. The platelet count returned to normal 5 weeks after discontinuing etretinate.

In May 1989 the patient was readmitted because of a relapse of the dermatosis and PUVA therapy was stopped. He was treated with high doses of steroids and etretinate 25 mg daily. On the fourth day of treatment with etretinate a marked thrombocytopenia developed, the platelet count being 90×10^9/l. A few days after stopping the drug the count rose to 136×10^9/l and was normal at subsequent follow-up visits.

We believe that the thrombocytopenia in our patient was related to etretinate therapy. There was a mild thrombocytopenia prior to treatment but this became much worse following retinoid therapy. We are aware of only one previous case of thrombocytopenia attributable to etretinate therapy[2] and one other case due to isotretinoin.[3] An additional case of cyclical neutropenia and thrombocytopenia with bone marrow findings suggesting myelodysplasia was recently reported[4] following treatment with isotretinoin. We suggest that monitoring of the platelet count during therapy is advisable in patients taking retinoids.

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References

1 de Groot AC, Nater JP. Dermatological drugs and cosmetics. In *Meyler's Side Effects of Drugs* (Dukes MNG, ed). Amsterdam: Elsevier, 1988; 284–315.
2 Liang R. Thrombocytopenia associated with etretinate therapy. *Acta Haematol* 1988; 79: 112–3.
3 Johnson TM, Rapini R. Isotretinoin-induced thrombocytopenia. *J Am Acad Dermatol* 1987; 17: 838–9.
4 Hughes BR, Cunliffe WJ. Development of folliculitis and pyoderma gangrenosum in association with abdominal pain in a patient following treatment with isotretinoin. *Br J Dermatol* 1990; 122: 683–7.

ADONIS 000709639100097U

Sclerotic (hypocellular) fibromas of the skin

Sir, Sclerotic or hypocellular fibromas of the skin have been reported[1,2] and can occur in patients with Cowden's disease.[3] We report seven patients with this tumour, two of whom had the characteristic lesions of Cowden's disease.

Seven patients (four males and three females, aged 34–64 years) each presented with a flesh-coloured solitary translucent papulonodular lesion that was round or oval and 5–10 mm in diameter. The lesions were symptomless and were located on the arms in four patients and one each on the nose, back and leg. The histological features were identical in all the seven tumours and consisted of a well-demarcated, non-encapsulated, dense proliferation of thick and hyalinized collagen which was localized in the upper and mid-dermis (Fig. 1). The collagen bundles were separated by prominent clefts that contained an increased amount of acid mucopolysaccharide as demonstrated by Alcian blue staining. A constant feature was the absence of elastic tissue in the lesions. Fibroblasts in the tumours were sparse and were absent in some areas. Immunohistochemical staining for S-100 protein and neurone-specific enolase in three of the cases and for desmine in four of the cases were all negative.

The clinical and histological features resembled those

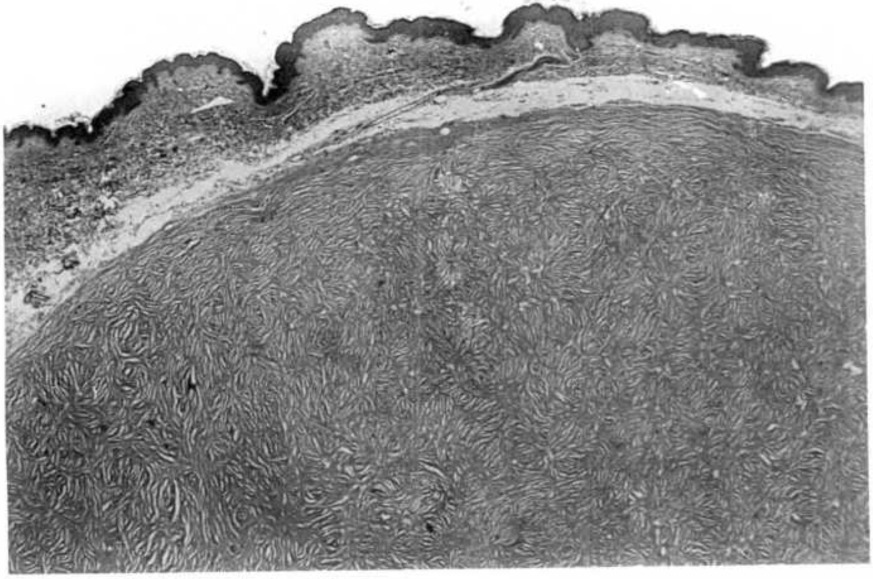

Figure 1. Nodule with hyalinized collagen, separated from the epidermis by a normal upper dermis.

previously described[1-3] and those reported as storiform collagenoma.[4] We believe that this sclerotic type of fibroma represents a unique hamartoma of fibrous tissue.

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References

1 Rapini RP, Golitz LE. Hypocellular fibromas. *J Cutan Pathol* 1986; **13:** 460.
2 Rapini RP, Golitz LE. Sclerotic fibromas of the skin. *J Am Acad Dermatol* 1989; **20:** 266–71.
3 Starink TM, Meijer CJL, Brownstein MH. The cutaneous pathology of Cowden's disease: new findings. *J Cutan Pathol* 1985; **12:** 83–93.
4 Maize J, Leidel G, Mullins S, Metcalf J. Circumscribed storiform collagenoma. *Am J Dermatopathol* 1989; **11:** 287.

ADONIS 0007096391000988

Purpura caused by UV-irradiation at 375 nm in a patient with hydroa vacciniforme

SIR, Actinic-induced purpura is not infrequent in sun-damaged skin of the elderly. However, in children solar purpura is rare and appears to be an unusual form of polymorphic light eruption.[1,2] We describe a young patient with hydroa vacciniforme (HV) who developed purpura at sites on her back irradiated with 375 nm monochromatic light.

A 10-year-old Japanese girl, at the age of 1·5 years developed bullae on the face and the backs of the hands following exposure to sunlight in the spring. Subsequently she has had itching of the skin following exposure to sunlight with erythema, oedema, papules and blisters. These lesions occurred in the summer and there was no family history of photosensitivity. On examination, there were areas of erythema with vesicles, erosions and crusts in the sun-exposed areas of skin (Fig. 1). Blood investigations and porphyrin studies were all normal.

Phototesting was carried out using a diffraction monochromator (JASCO CRM-FM, Tokyo, Japan) with a 5 kW xenon arc lamp.[3] No vesicles developed after irradiation at 310 or 390 nm on 5 consecutive days with a daily dose of $6{\cdot}6 \times 10^2$ J/m^2 and

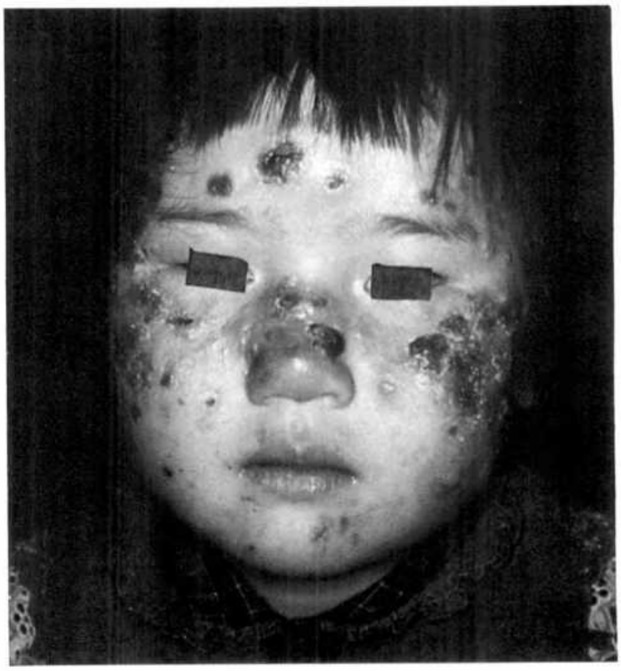

Figure 1. Erythema, bullae, crusts, and pitted scars on face of patient aged 4 years.

$2{\cdot}6 \times 10^4$ J/m², respectively. At 300 nm 2 consecutive days of treatment at a daily dose of $6{\cdot}6 \times 10^3$ J/m², which was about 60 times greater than her MED, produced a vesicular reaction which healed without scarring. At 330 nm a daily dose of $1{\cdot}5 \times 10^4$ J/m² over 3 consecutive days gave rise to hydroa vacciniforme lesions. Similarly, at 360 nm 2 consecutive days of treatment with a daily dose of $4{\cdot}4 \times 10^4$ J/m² produced similar lesions, and a biopsy of one of these showed epidermal spongiosis and necrosis of the underlying dermis as seen in HV.[3] These lesions healed with scarring.

Exposure of the back of the patient to a wavelength of 375 nm for 5 min at a dose of $5{\cdot}5 \times 10^3$ J/m², produced no response but 11 min of irradiation with $1{\cdot}1 \times 10^4$ J/m², produced purpura within 24 h. This was repeated on another site and produced the same response. Other wavelengths used, 300, 310, 330, 360 and 390 nm, did not induce the purpuric response. As in a previous report[4] we found that the HV lesions were caused by UVA and less energy was required to induce the lesions at 330 nm than at 360 nm.[3] Exposure to 330, 340 and 350 nm have been reported to cause the development of skin lesions in HV.[5]

Although there are many factors that can produce purpura,[6] these were excluded in our patient either from the history or from the laboratory investigations. It appears that in our patient purpura induced by monochromatic light at a wavelength of 375 nm affected and damaged the dermal blood vessels. It is not clear whether there is a relationship between the vascular damage caused by this wavelength and the pathogenesis of the lesions of HV. We cannot explain why our patient never had purpura following sun exposure, though testing her with monochromatic light at 375 nm produced purpuric lesions.

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References

1 Kalivas L, Kalivas J. Solar purpura. *Arch Dermatol* 1988; **124**: 24–5.
2 Ros A-M. Solar purpura: an unusual manifestation of polymorphous light eruption. *Photodermatology* 1988; **5**: 47–8.
3 Kunieda M, Sato K, Hayakawa K, Kitano Y. Successful induction of hydroa vacciniforme lesions with monochromatic ultraviolet light. *Jpn J Dermatol* 1985; **95**: 893–9.
4 Goldgeier MH, Nordlund JJ. Lucky AW *et al.* Hydroa vacciniforme: diagnosis and therapy. *Arch Dermatol* 1982, **118**: 588–91.
5 Sunohara A, Mizuno N, Sakai M *et al.* Action spectrum for UV erythema and reproduction of the skin lesions in hydroa vacciniforme. *Photodermatology* 1988; **5**: 139–45.
6 Leung AKC Purpura associated with exposure to sunlight. *J Roy Soc Med* 1986; **79**: 423–4.

ADONIS 000709639100099M

British Journal of Dermatology (1991) 124, 398–400.

Book Reviews

Sexually Transmitted Diseases. T.F. MROCZKOWSKI (1990). New York: Igaku-Shoin Medical Publishers Inc. Pp. 404. Illustrations: 176. ISBN 0-89640-1-163-4. Price £96.00.

This American book is the first volume in a series entitled '*Topics in Clinical Dermatology*'. In the U.S.A., patients with sexually transmitted diseases may be seen by gynaecologists, urologists, general physicians and quite frequently dermatologists, unlike in the U.K. where we have the well established specialty of Genito-Urinary Medicine, with the well organized clinics. However, the topic is appropriate for us because in 1992 the U.K. and Republic of Ireland will be part of the European Community where dermatology and venereology is regarded by most member countries as one specialty. Almost certainly training in the U.K. in both these specialties will overlap considerably in the future.

The book itself is full of detail regarding diagnostic methods, aetiology, epidemiology, clinical presentation, differential diagnoses and treatment. It is also well referenced. However, the layout is not very logical. Infection with chlamydia is certainly very common, but there are few skin manifestations and therefore an odd choice for Chapter 1. While discussing the diagnosis of chlamydia, the author refers the reader to Chapter 2 where detailed sampling techniques for gonorrhoea are described. Other chapters deal with such topics as molluscum contagiosum and herpes and it is not until Chapter 8 that syphilis, and Chapter 14, that AIDS are mentioned, both of which certainly have many dermatological presentations. Included in the text are good chapters on some tropical diseases.

There are a lot of black and white and colour pictures, which presumably accounts for the high cost of this book. These pictures are of varying quality, but generally poor.

At the cost of £96 there are cheaper and more suitable textbooks on sexually transmitted diseases available for the U.K. market.

JANE E.ROBERTS

Clinical Dermatology: A Colour Guide to Diagnosis and Therapy. T.P. HABIF (1990). St Louis: CV Mosby Company. Pp. 756. Illustrations 875.

This is the second edition of this well-illustrated textbook. Like its predecessor it aims to meet the needs of several widely different groups of readers—medical students, interns, general physicians and dermatologists. It covers all aspects of dermatological disease and available treatments. The layout and chapter headings are similar to those in the well-established dermatology textbooks. Aetiological factors and pathophysiology are dealt with briefly and the major part of each chapter deals with clinical features and management.

The chapters on cutaneous infection, connective tissue diseases and bullous diseases are particularly good. The style throughout is very readable and the author uses tables to clarify information like frequencies of various autoantibodies in connective tissue problems. This will endear the book to medical students and also perhaps to their harassed clinical teachers. Each chapter has an up-to-date extensive bibliography. Clinical photographs are generously spread throughout the book and are of extremely high quality. In addition there are several excellent line drawings including a 'how to do it' section on minor surgical procedures.

The major part of each chapter is given over to the clinical features and treatment of the diseases. The treatment schedules are fairly detailed giving durations, dosages and possible complications. Drugs are described by both their generic and proprietary names which helps the British reader considerably. The author includes an exhaustive list of topical steroids arranged in potency groups and a separate dermatological formulary for easy access to drug details.

The language and suggested approaches to treatment are much more American than British. Unfortunately no concessions to European treatments experience or publications are made whatsoever which may detract from its overall value. Despite this, I feel that this book would be a useful addition to the library of any dermatology department though it would never replace the established general textbooks.

V.R.DOHERTY

Eicosanoids and the Skin. Edited by T.RUZICKA (1990). Boca Raton: CRC Press. Pp. 215. ISBN 0-8493-6032-3. Price £80.

This book is aimed at basic researchers interested in the role of eicosanoids (arachidonic acid and its metabolites) in inflammatory and proliferative skin disease, cutaneous carcinogenesis, wound healing, immune regulation, and as dermatological targets for anti-inflammatory therapy. The book is in five sections dealing with (a) general aspects, (b) the role of arachidonic acid metabolism in skin disease (c) the biological and biochemical effects of arachidonic acid metabolites in skin, (d) inhibitors of arachidonic acid metabolism and (e) a brief summary of new concepts. Each section, excluding the last, contains between two and five detailed chapters.

The editor has succeeded in recruiting an appropriate international panel of expert contributors who have on the whole produced up-to-date, well-balanced, informative chapters which include useful lists of references—between 18 and 194 (mean 84) per chapter. Most chapters are in the form of reviews of variable length with the exception of Chapter 7 which deals with the isolation of a 5-HETE-specific chemotaxis inhibitor in psoriatic lesional scales. This chapter is more in the form of an original paper dealing with a single, narrow topic and therefore differs in structure from most of the other chapters. Chapter 11 was also rather short and narrowly focused, dealing with the effects of certain hydroxy fatty acids on fibroblast chemotaxis.

Eicosanoids have occupied the minds of enormous numbers of researchers for at least two decades, although in comparison with other disciplines, dermatological researchers in this area have formed a relatively small group. This research has not yet had much impact on clinical dermatology, although work over the next 5 years should reveal whether pharmacological manipulation of eicosanoids has any therapeutic potential in skin disease. It is now entirely appropriate that a book should be produced reviewing the work done mainly over the last 10 years. I am not aware of any publication which competes with this volume, but at £80 it is expensive and therefore unlikely to be bought by individual researchers. However, it deserves to be in libraries serving academic dermatology departments and would be a valuable reference for non-dermatological eicosanoid biologists interested in the skin, whether in industry or academia.

R.D.R.Camp

Immunodermatologie: Pathogenese—Diagnostik—Therapie. Edited by Hans-Dieter Göring and Ludwig Raith (1990). Leipzig: VEB Georg Theme. Pp. 272. Illustrations: 43. ISBN 3-7404-0102-8.

In recent years there have been an increasing number of books published on the theme of immunology and the skin. This is not surprising as there have been great advances in our understanding of immunological mechanisms behind commonly observed skin disorders. The developments in this field are rapidly changing and up-to-date information is essential. The two editors here are both from the part of Germany which used to be known as the German Democratic Republic. Twelve co-workers have contributed to the four chapters of the book.

The four main sections of the book have as their headings Selected investigation methods; immunological aspects of dermatologically relevant disorders; immunotherapy and allergic reactions to naturally occurring chemicals. The information presented in the book is rather uneven but the section on complement disturbances and the discussion of the various methods of investigation of macrophage function are quite well written.

Some of the comments on the clinical disorders where immune factors are thought to be important are rather unusual. For instance, in Chapter 2, Dr Göring separates so-called circumscribed scleroderma into 13 distinct forms. Most clinicians and investigators would feel there is a good degree of overlap between these different forms. Later in the same chapter the course of atopic eczema is illustrated in a table detailing 11 different variations. This sort of analytical approach again seems rather bogus and unhelpful. Although epidermolysis bullosa acquisita is mentioned, the information with regard to the EBA antigen is not as up to date as it might be.

Although for the German reader there are some good contributions to this book, the overall impact is that of a rather uneven and slightly out of date introduction to immunodermatology and I would feel that most individuals might find clearer and more succinct information in other sources.

N.Smith

Differential Diagnosis in Dermatology. R.Ashton and B.Leppard (1990). Oxford: Radcliffe Medical Press. Pp. 300. ISBN 1-870905-21-0. Price £49.50.

A novel approach to the dermatology textbook has been applied in this book aimed at primary care physicians faced with the problems of dermatological diagnosis in their busy surgery. The first chapter introduces the important historical points and physical signs to be elicited prior to using the subsequent chapters. These chapters are divided by site of the problem and subdivided on basis of history and clinical features. Each chapter consists of three parts, the algorithms, clinical pictures and text which are to be used together to arrive at the diagnosis. This task is simplified by the use of individual chapter indexes which allow rapid reference to the relevant section of the chapter. The algorithms are easy to follow, refer to other sections when needed and combine with the text and clinical photographs very well. The clinical photographs of which there are many are particularly worthy of mention as they are of excellent quality and all in colour. A certain amount of repetition is found with this format but in the context of the designed use of this text, as a rapid reference rather than as a conventional textbook I do not see this as a problem. One area that is notably missing from this book is therapy although I understand that another book is planned in the series with this aspect in mind.

I am sure that general practitioners will find this book extremely useful when faced with a dermatological diagnostic problem and will gain confidence in their diagnostic acumen due to the accessibility of the information and support of these excellent clinical photographs. However, they will need to spend a little time familiarizing themselves with the book, particularly the first chapter on history and examination and then require a reference on therapeutics and management.

W.Perkins

Problematic Pigmented Lesions. A case method approach. M.C.Mihm, Jr. and P.B.Googe (1990). Philadelphia: Lea & Febiger. Pp. 543. ISBN 0-8121-1261-X.

This is an interesting book. The starting point is the collection of 'problem' slides, some local, some referred, which all specialist pathologists have in a drawer, box or cupboard and draw on to illustrate lectures. The authors of this book have taken this a step further and turned them into a book. There are 16 chapters, 15 dealing with specific melanocytic pathology problems such as the congenital naevus and its variations, vulvar naevus with atypism, primary versus metastatic melanoma and similar difficult areas. After a brief introduction the chapter topic is expanded on by an individual case with the referral information, the report given, and an explanation or comment on the report. There are from four to eight illustrations in black and white accompanying this text. There are also, tucked away at the end of the book, 20 pages of rather small colour illustrations duplicating a proportion of the black and white plates. They are not referred to in the body of the text so it is necessary to dip into the back to see if a colour illustration is available. A problem with this book is that overall the quality of the black and white illustrations is variable. This may well be explained by the problem of photographing slides sent from other centres, but now that the dermatopathological eye has acclimatized to the excellent black and white or colour reproduction offered by Bernard Ackerman or Philip McKee, less than excellent black and white illustrations are a disappointment.

I liked the layout of the book and found the problems encountered very similar to those seen in Glasgow, but did not always agree with the opinions offered. Specifically, I felt that the authors had a rather broad concept of the pigmented spindle cell naevus and included in this category naevi I would have considered to be Spitz lesions. This is only of academic rather than patient management significance, but a second, rather more profound difference was in the authors' interpretation of the minimal deviation melanoma which they are happy to diagnose with no overlying junctional activity. The fact that the book is only generally referenced at the end and that there are no specific references in the text made it difficult to take such disagreements further.

In summary, this book is a good idea in its layout but could be improved on in subsequent editions. The content is probably most useful for those who already have a considerable knowledge of the difficulties of melanocytic pathology. They will find it both interesting and at times controversial.

RONA M.MACKIE

British Journal of Dermatology (1991) **124**, 401.

News and Notices

18th World Congress of Dermatology

12–18 June 1992, New York, U.S.A.

The 18th World Congress of Dermatology will take place in New York City on 12–18 June 1992. To encourage the participation of young dermatologists, the Congress will award a limited number of scholarships.

Dermatologists from Developing Countries. Applicants must be no older than 38 years of age at the time of the Congress. The scholarship will provide complimentary registration and hotel accommodations (two awardees to a room) and a moderate subsistence allowance. The awards are competitive and are contingent on sponsorship by the applicant's national society. Abstract submission is mandatory. Further information and application forms can be obtained from your national society before 1 June 1991.

Dermatologists from Developed Countries. Applicants must be a Resident or Fellow in a full-time training programme. The scholarship will provide complimentary registration and a small subsistence allowance. A letter from the educational or training institution validating the applicant's status must be submitted with the application form. Abstract submission is mandatory. The forms are available from: 18th World Congress Secretariat, 875 Kings Highway, W Deptford, NJ 08096, U.S.A.

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JOURNAL OF MEDICAL AND VETERINARY MYCOLOGY

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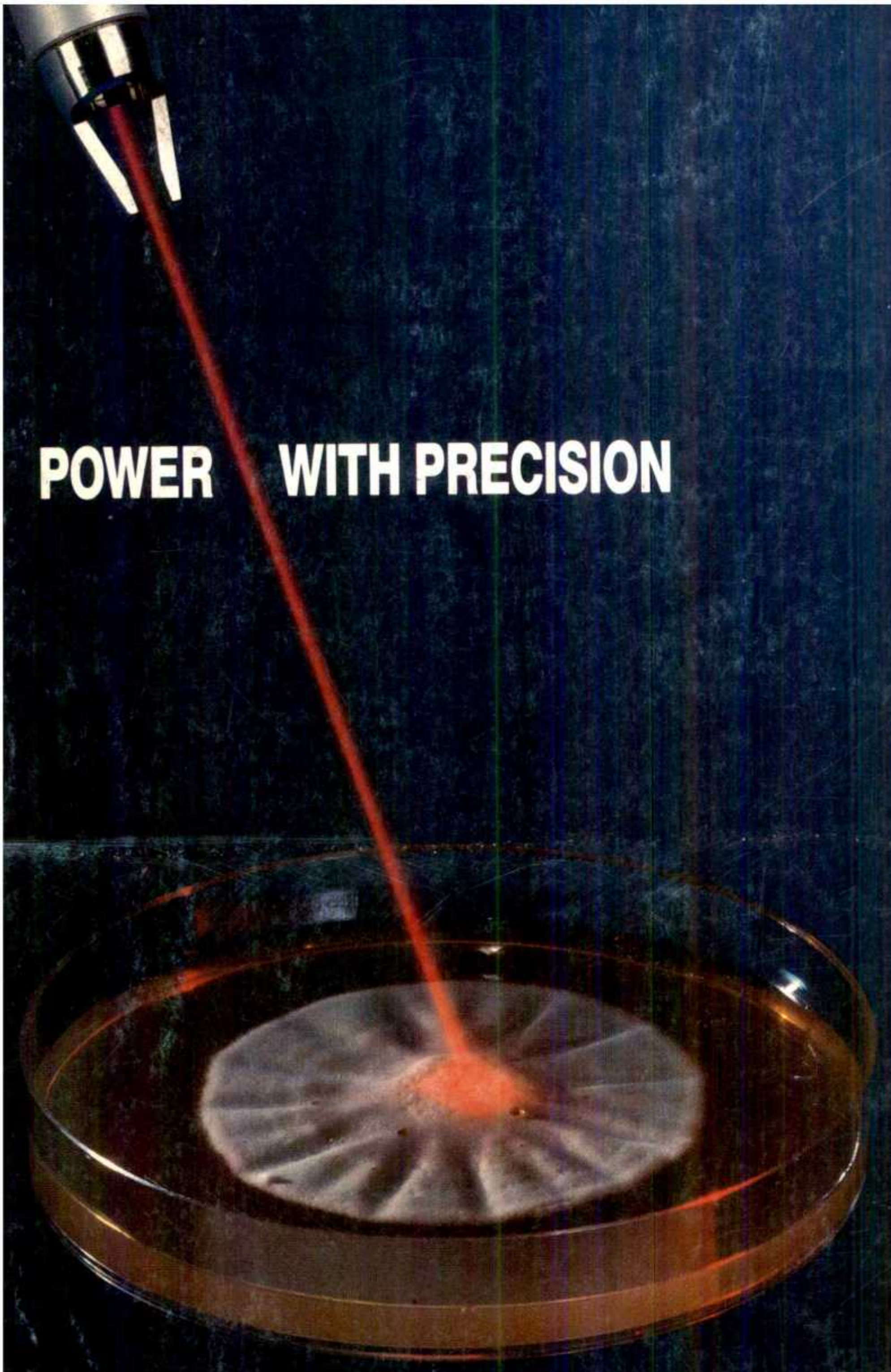

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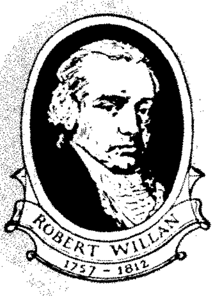

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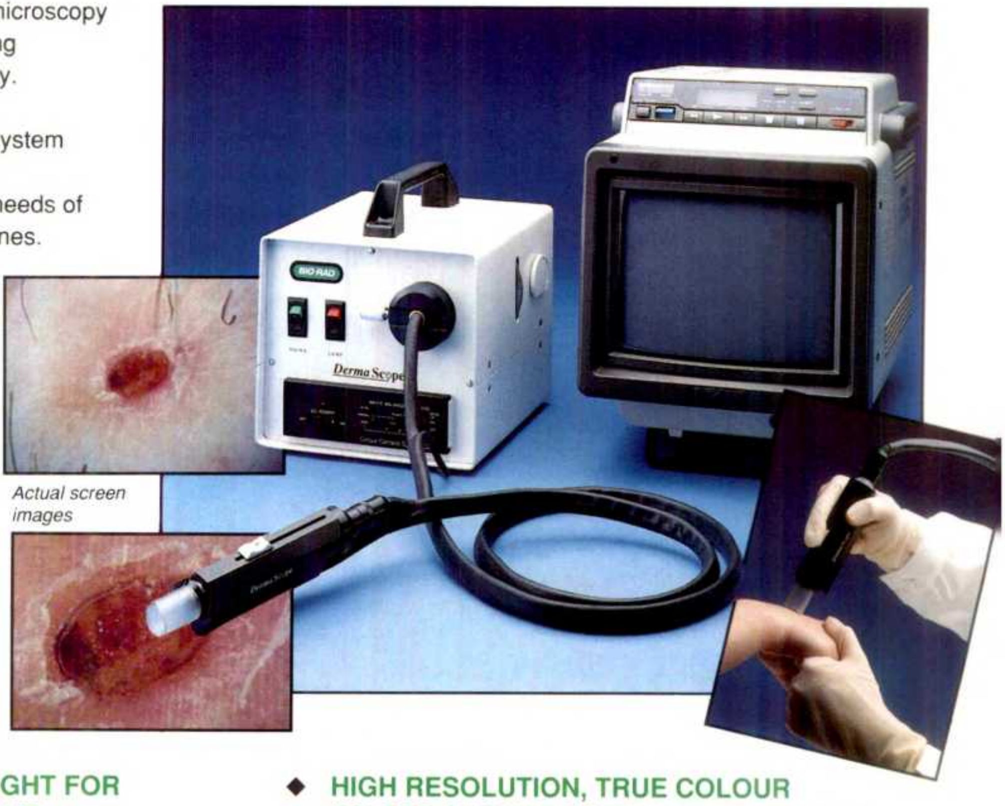
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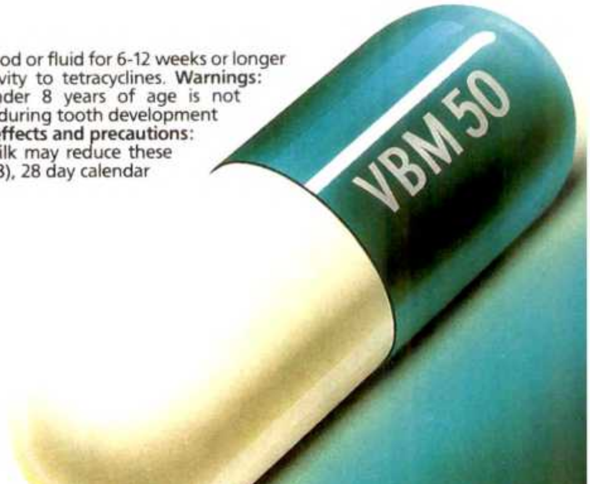

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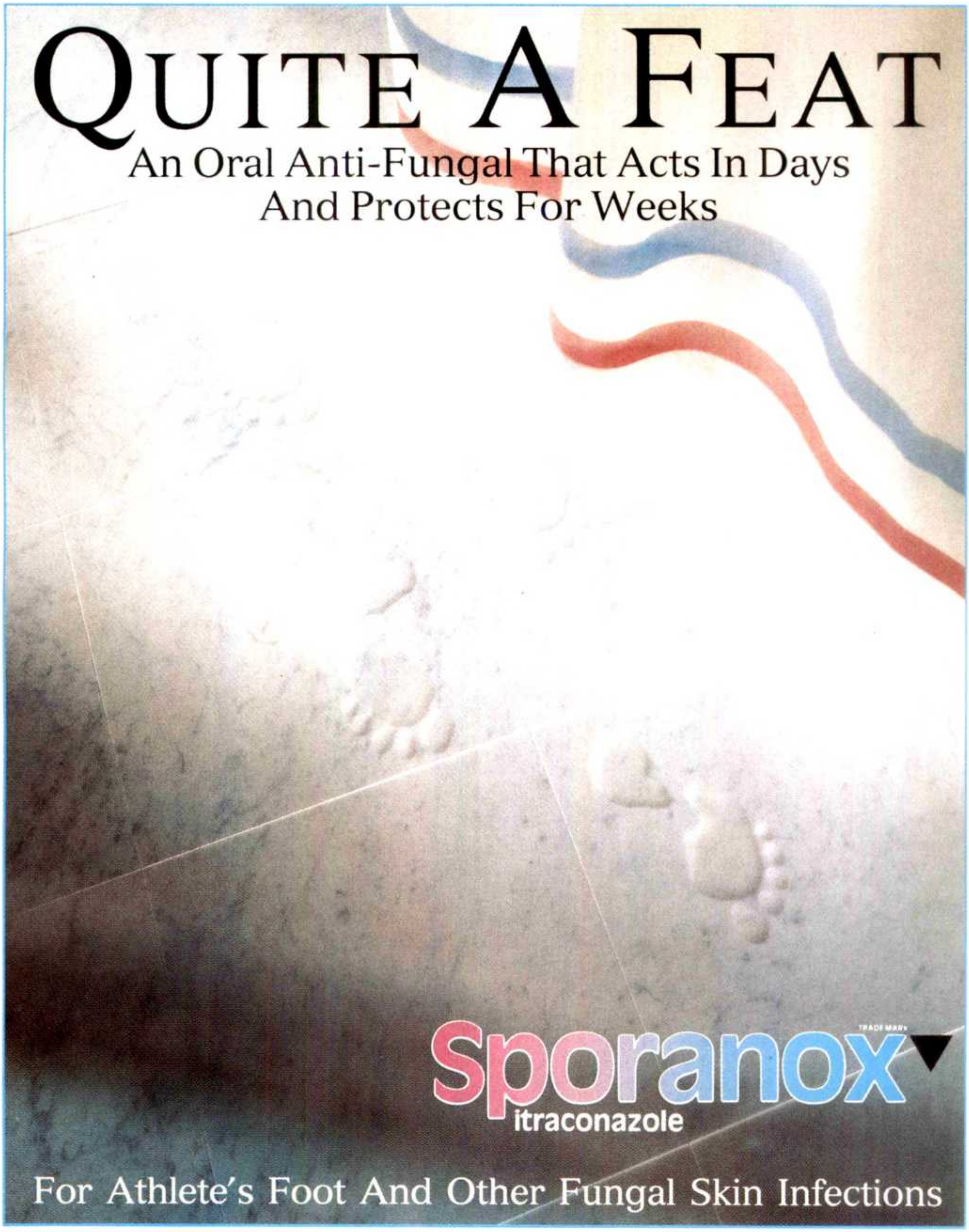
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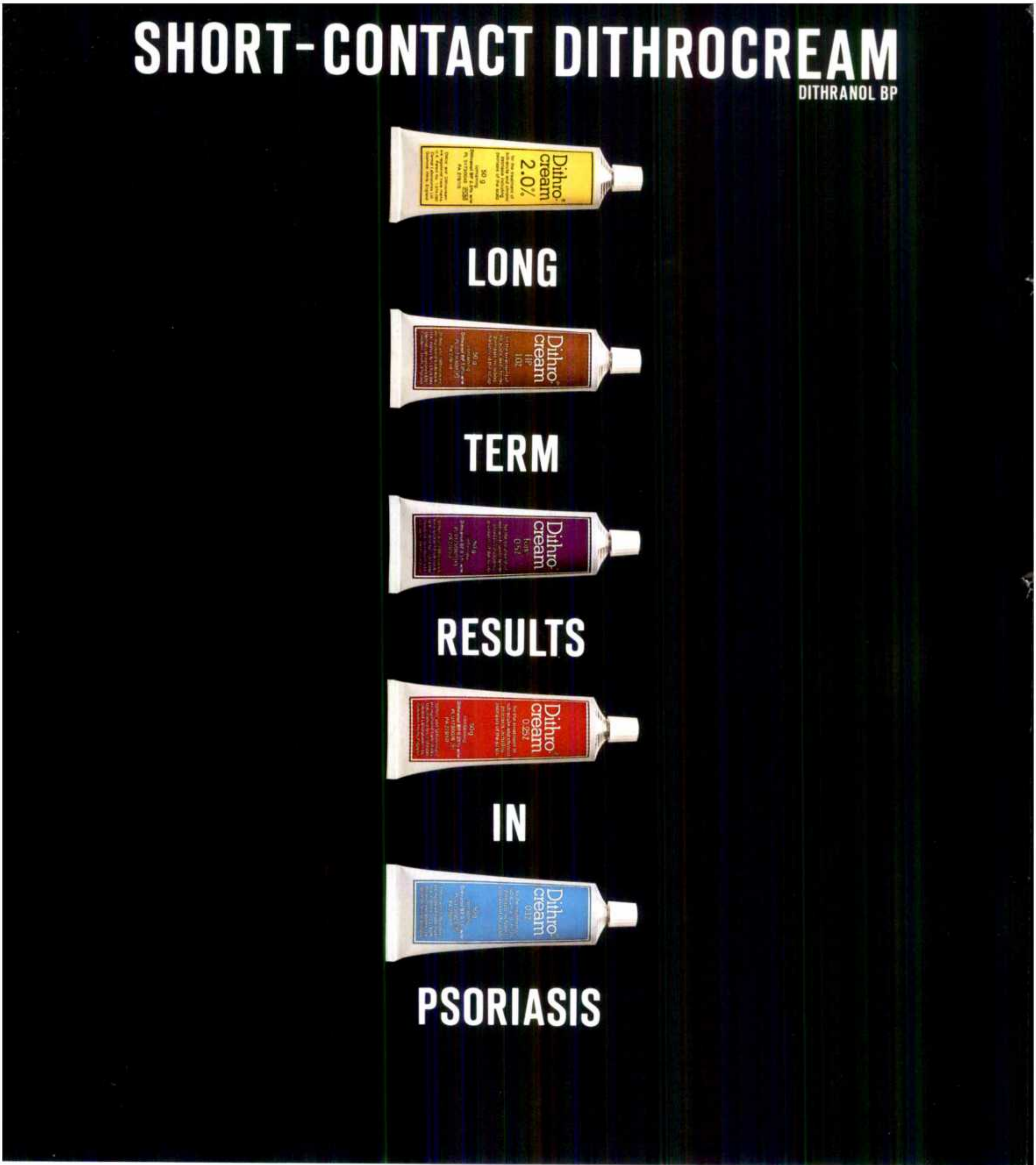

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Morphological evidence for calcium-dependent association of calgranulin with the epidermal cytoskeleton in inflammatory dermatoses

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Summary The association of calgranulins, intracellular calcium-binding proteins, with the keratinocyte cytoskeleton has been studied. These molecules are expressed in various inflammatory dermatoses and in organ-culture explants. Triton X-100 extraction in the presence of calcium or EDTA suggested that calgranulins are detergent insoluble in the presence of calcium. The molecules were localized in a plaque-like structure at the cell periphery in lesional skin and in organ-culture explants. Following induction of calgranulins *in vitro* there was a redistribution of the intermediate filament cytoskeleton into a perinuclear halo, although desmosomes remained intact. These various features suggest that these members of the S-100 protein family have a role in cytoskeletal changes seen in various skin diseases.

Calgranulins are intracellular calcium-binding proteins which are expressed in keratinocytes in various inflammatory dermatoses.[1–3] Two calgranulin molecules have been identified. Calgranulins A and B are polypeptides of 11 kDa and 14 kDa, respectively, and exist intracellularly as a non-covalently bound heterodimer.[4] They were initially identified as myeloid differentiation markers.[5,6] The genes encoding both calgranulins have been cloned and sequenced. Both genes are located on chromosome 1 and by gene sequence homology and exon/intron-structure have been classified as belonging to a family of intracellular calcium-binding proteins.[4,7] Other members of the family include S-100 α and β proteins, calcyclin (2A9), intestinal calcium-binding protein (calbindins) and the p11 regulatory subunit of lipocortin II/calpactin I. Although the calcium binding site is of the E–F hand structure, these proteins show no other homology with calmodulin and are therefore considered to be a distinct family. The several members of this family have been shown to be important in intracellular calcium sequestration, the regulation of phospholipase A_2 inhibition by lipocortin and binding to growth factor receptor tyrosine kinase substrates.[8] These calcium binding proteins also exhibit molecular association with different parts of the cytoskeleton of the cells in which they are found[9] and with cytoskeletal extracts in gel-overlay experiments.[10]

We have previously shown that these molecules are induced in lesional keratinocytes over a period of 24 h[2] and can be induced in keratinocytes *in vitro* over a similar time course.[11] Calgranulin expression in these *in-vitro* experiments occurred independently of other markers of cutaneous inflammation and was independent of cell proliferation but required *de novo* mRNA and protein synthesis.[11]

In this paper we have examined the association of epidermal calgranulins with the cytoskeleton of keratinocytes in inflammatory dermatoses as seen in other members of the S-100 protein family in other tissues. We have also investigated the calcium dependency of cytoskeletal binding of calgranulin and described the structural alterations of intermediate filaments which accompany calgranulin expression.

Methods

Explant culture

Skin specimens were obtained from redundant skin at reduction mammoplasty. Discs of skin, 2 mm in diameter, were immersed in tissue culture fluid (Glasgow's modification of Eagle's medium supplemented with 10% fetal calf serum and containing 100 IU/ml penicillin and

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50 μg/ml streptomycin) in a Petri dish and cultured at 37°C in 95% air/5% CO_2 for up to 48 h.

Immunocytochemistry

Skin biopsies from inflammatory dermatoses and tissues from *in vitro* normal skin explants were snap frozen in OCT in liquid nitrogen and stored at -70°C until use. We and others have documented the range of inflammatory dermatoses diseases in which calgranulin is expressed,[2,3] so we chose a smaller number of cases from selected diseases for inclusion in this study (Table 1).

Sections 5-μm thick were cut, mounted on glass slides and briefly fixed in cold acetone. Calgranulins were detected by the monoclonal antibodies MAC 387, CF145 and CF557, the specificities of which have been described previously.[4,5] Other antibodies to cell surface and cytoskeletal components were also used and are described in Table 2. Primary antibody binding was visualized by indirect immunofluorescence or indirect immunoperoxidase methods as described previously.[11]

Microscopy

The immunofluorescence preparations were examined using Zeiss confocal laser scanning microscopy (CLSM). This is a recent advance in light microscopy[12] which provides digitally recorded high-resolution images with out-of-focus blur removed. In addition the image generated is from a depth of field less than 1 μm even in specimens which are considerably thicker. We have previously used this technique to identify basement membrane micro-fragmentation and cytoskeletal changes in early lesions in bullous dermatoses.[13]

Table 1. Selected dermatoses studied by immunofluorescence

Bullous pemphigoid	8 cases
Epidermolysis bullosa acquisita	1 case
Linear IgA disease	3 cases
Pemphigus vulgaris	2 cases
Lichen planus	2 cases
Normal skin	5 cases

Table 2. Specificity of antibodies used in this study

Antibody	Specificity
MAC 387	Calgranulin B
CF 557	Calgranulin B
CF 145	Calgranulin A
DF 1516	p105, basal keratinocytes
LP 34	Cytokeratin
Desmoplakin I and II	Desmosomal plaque protein

Western blotting

Two extraction buffers were used in a method previously described.[11] The detergent-based extraction buffer consisted of 8% (w/v) sodium dodecyl sulphate (SDS) in 50 mM Tris-HCl containing 10 μM phenyl methyl sulphonyl fluoride (PMSF), 10% (v/w) 2-mercaptoethanol, 4% (v/v) non-idet P-40 and 0·025% (w/v) 2-macroglobulin adjusted to pH 7·4. The urea-based extraction buffer was 8 M urea in 50 mM Tris-HCl containing 10 mM PMSF, 2·5 mM EDTA, 10% 2-mercaptoethanol and 0·025% 2-macroglobulin, also adjusted to pH 7·4. The electrophoresis sample buffer contained 5% (w/v) SDS in 50 mM Tris-HCl containing 10 mM EDTA, 10% 2-mercaptoethanol and 0·04% (w/v) bromophenol blue. For separation of urea-extracted tissue, urea to a final concentration of 8 M was added to this buffer.

Three tissue samples from psoriatic scale and two from bullous pemphigoid epidermis were used. The samples were homogenized and sonicated (12 kHz) at 4°C in TBS containing 10 mM PMSF. The samples were extracted using the detergent-based extract and the detergent-soluble fraction was collected. The detergent-insoluble residue was then extracted with the urea-based extract and this fraction was collected. Both fractions were then analysed by Western blotting.

Gel electrophoresis was performed with the Phast gel system using 12·5% homogeneous precase polyacrylamide gels. In later experiments 5–25% gradient gels were also used.

The separated proteins were transferred to nitrocellulose paper and free-protein-binding sites were blocked. Strips of nitrocellulose paper were incubated with primary antibodies. Antibody binding was visualized by an avidin-biotin alkaline phosphatase-labelled anti-mouse detection system (Amersham). The Western blots were examined visually and by LKB Ultrascan laser densitometry. The M_r of reactive bands was calculated by using prestained M_r markers in the SDS-PAGE.

The antibodies used in the Western blotting experiments were MAC 387 (1/10) to detect calgranulin, LP34 (1/10) to confirm reactivity with the appropriate cytokeratins and anti-α feto protein (1/10) as a control.

Cytoskeletal extraction

Tissue from 2-mm diameter skin biopsies of lesional skin or from the tissue-culture explants was extracted in detergent buffer for 20 min. The extraction buffer consisted of Triton X-100 0·2% in PBS with either 2 mM $CaCl_2$ or 2 mM EDTA. The insoluble tissue fragments were then fixed and processed for immunocytochemistry.

Electron microscopy

Pieces of tissue culture explants 1 mm in diameter were fixed by immersion in 3% glutaraldehyde in 0·2 M sodium cacodylate pH 7·4 for 2–4 h at 4°C. Tissues were post-fixed in 1% osmium tetroxide for 24 h. Tissue was embedded in araldite, 90-nm sections cut, mounted on copper mesh grids and stained with uranyl acetate and lead citrate. Ultrathin sections were viewed on a JEOL 100s transmission-electron microscope.

Results

Intracellular localization of calgranulin

Reactivity for p11 and p14 calgranulin was seen in the keratinocytes of lesional skin and in the skin explants as previously described.[11] Staining using indirect immunofluorescence to detect the calgranulins in frozen sections and visualization by high-resolution confocal laser scanning microscopy showed punctate staining at the cell periphery (Fig. 1). This pattern was seen in both lesional skin and in organ-culture specimens (24 h in culture). A similar localization in both tissue types was seen in sections stained with antibody to desmoplakin I and II (Fig. 2).

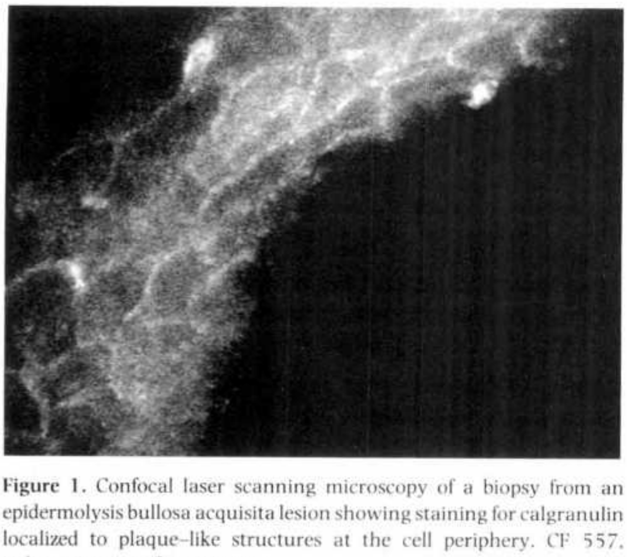

Figure 1. Confocal laser scanning microscopy of a biopsy from an epidermolysis bullosa acquisita lesion showing staining for calgranulin localized to plaque-like structures at the cell periphery. CF 557, indirect immunofluorescence.

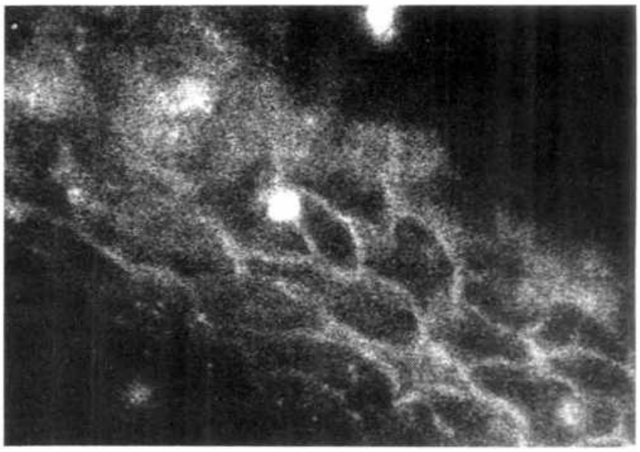

Figure 2. A section adjacent to that in Figure 1 showing the plaques of desmosomes stained with a monoclonal antibody to desmoplakins.

The binding of calgranulins to detergent-insoluble cytoskeletal components was investigated by Triton extraction in the presence or absence of calcium. Sections from tissue extracted by Triton X-100 in the presence of Ca^{2+} showed immunoreactivity for calgranulin whereas tissue extracted in the presence of EDTA was negative (Fig. 3). Adequate extraction in the Triton X-100/Ca^{2+} method was confirmed by the loss of the epidermal cell membrane antigen detected by DF 1516.

Western blotting

The Western blotting experiments confirmed that the molecules detected by antibodies CF145, CF557 and MAC387 in lesional keratinocytes had M_r of 11 kDa, 14 kDa and 14 kDa, respectively (Fig. 4). This is identical to the M_r of the calgranulins extracted from leucocytes. LP34 showed reactivity with several cytokeratin bands between M_r 45 kDa and 60 kDa. The antibody to desmoplakin I and II reacted with bands of M_r 210 kDa and 240 kDa.

Cytoskeletal reorganization and calgranulin expression in tumour culture explants

Electron microscopic examination of skin explants was performed on tissue sampled at 0, 6 and 24 h in culture. The morphology at 0 and 6 h was that of normal epidermal keratinocytes. However at 24 h, by which time calgranulin expression was confirmed, there was a change in the keratinocyte cytoskeleton. Bundles of intermediate filament (10-nm diameter) were seen in dense masses surrounding the nucleus (Fig. 5a). Desmosomes remained intact and some intermediate filaments

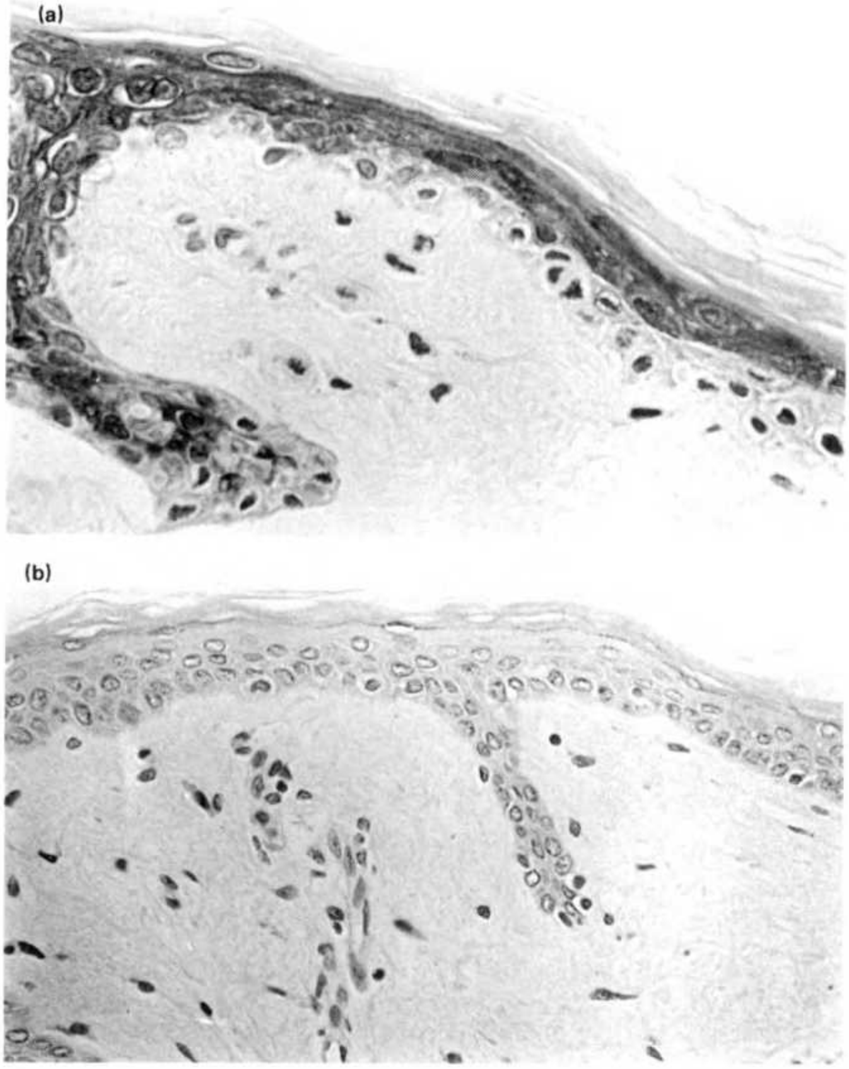

Figure 3. MAC 387 staining of organ culture explant extracted by Triton X-100 in the presence of (a) Ca^{2+} and (b) EDTA. Reactivity is seen in the epidermis only in (a). MAC 387, organ culture explant 24 h, indirect immunoperoxidase.

were seen inserted into the desmosomal plaques (Fig. 6). The epidermal cells from tissue explants contained pinocytotic vesicles. Immunofluorescence staining with LP34 showed strong fluorescence in a perinuclear distribution with separate peripheral cytoplasmic reactivity in explants after 24 h *in vitro* (Fig. 5b). At culture times of 0 and 6 h more diffuse cytoplasmic reactivity was seen.

Discussion

Calgranulins are calcium binding proteins which are found in keratinocytes in lesional skin from a variety of inflammatory dermatoses but which are not present in normal epidermis.[1-3] We have previously shown that calgranulin A and B are simultaneously co-expressed, that the keratinocytes synthesize calgranulins rather than absorption of protein secreted by inflammatory cells and that their expression in inflammatory dermatoses requires *de novo* mRNA transcription and protein synthesis in keratinocytes.[11] We have studied the cytoskeletal association of calgranulins in keratinocytes and have found evidence for the calcium dependency of this interaction.

We have used the criteria of detergent insolubility and co-localization with a known cytoskeletal component to define the cytoskeletal association of calgranulins. These criteria have previously been widely accepted.[9,14] Osborn *et al.*[9] have shown that the calgranulin homologue p11, the regulatory subunit of lipcortin II, was associated with the cytoskeleton. They have shown for p11, as we have for calgranulin, that the detergent

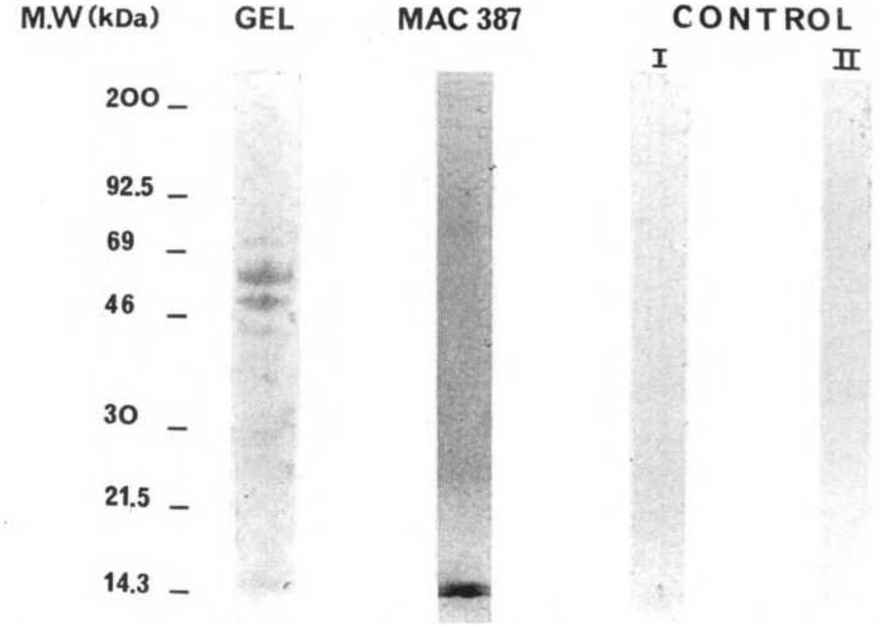

Figure 4. Western blot of urea-based extract from lesional keratinocytes. MAC 387 detects a band of M_r 14 kDa. Control experiments of normal skin extract (I) and use of an irrelevant antibody on lesion skin extract (II) show no reactivity.

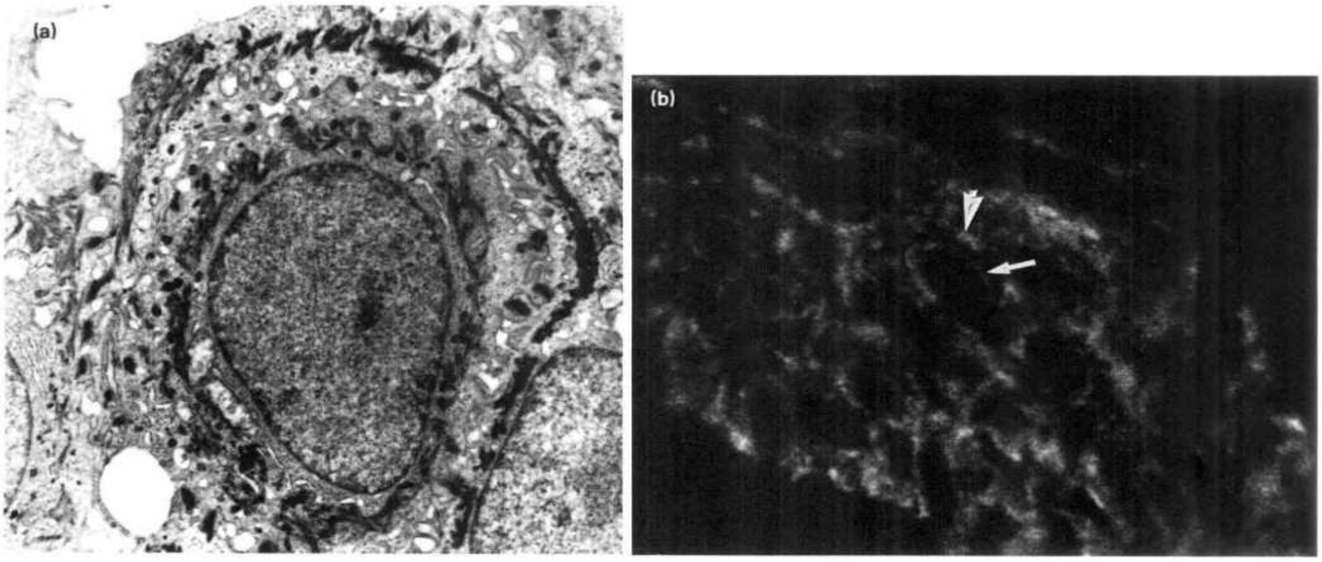

Figure 5. (a) Electron micrograph of cell from the immediate suprabasal layer of the epidermis of a tissue explant cultured for 24 h during which calgranulins were induced. There is collapse of the intermediate filament network into a perinuclear halo but desmosomes remain intact. (b) Frozen sections of similar tissue stained with LP34 to detect cytokeratin viewed by confocal laser scanning microscopy shows reactivity in a perinuclear halo (arrow) and some reactivity at the cell periphery (arrowhead).

insolubility of these calcium-binding proteins was dependent on the presence of calcium ions in the extraction medium. Both p11 and calgranulin became detergent soluble when EDTA was added to the detergent extraction buffer.

In the frozen-section specimens the calgranulins were localized in plaque-like structures at the cell periphery. This cellular distribution was found in both lesional skin and tissue culture explants. Several different types of plaque-bearing junctions, which are part of the cell adhesion-cytoskeleton complex, are found in the skin. Hemidesmosomes are found in basal keratinocytes[15] but

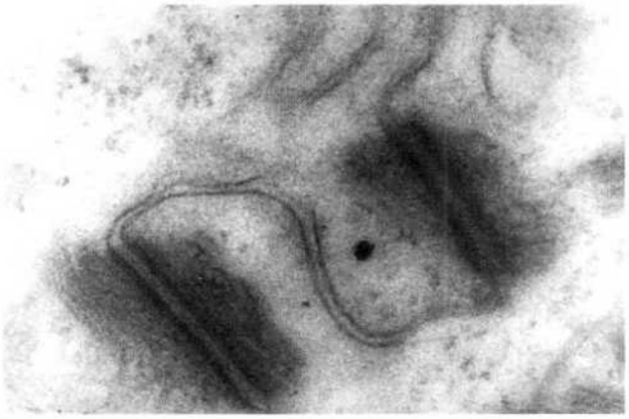

Figure 6. Electron micrograph of intact desmosomes after the induction of calgranulins *in vitro*.

calgranulins are poorly expressed by these cells.[11] Desmosomes and zonulae adherentes both contain electron-dense plaques and are found in keratinocytes.[16] In our experiments the proteins of desmosomal plaques and desmoplakins 1 and 11 showed a similar distribution to calgranulin. Ultrastructurally desmosomes were normal in the tissue culture specimens although zonulae adherentes were poorly represented. Wilkinson *et al.*[4] also noted plaque-like reactivity for calgranulin in oral mucosa and psoriatic epidermis.

In the organ culture explants on immunocytochemistry keratin reactivity became condensed into a perinuclear halo. This observation was confirmed by electron microscopy which showed that bundles of intermediate filaments were concentrated around the cell nucleus. A similar redistribution of intermediate filaments is seen in keratinocytes cultured in low calcium conditions.[17]

In formalin-fixed tissue a more diffuse pattern of calgranulin distribution is seen.[1–3] However, this fixative does not contain calcium ions and calgranulin may dissociate from its plaque site. Furthermore it has previously been noted that in formalin-fixed tissue desmosomal protein and glycoproteins may show a diffuse cytoplasmic distribution on immunocytochemistry.[18,19]

The significance of calgranulin expression and its calcium-dependent localization in the detergent-insoluble plaques is not known. Several regulatory molecules and protein kinases including src pp60 are present within junctional plaques.[20,21] The major substrate for C-SRC tyrosine kinase is p36,[22] now known to be the heavy chain of lipocortin II.[8] The regulatory subunit of lipocortin II, p11, is extensively homologous with calgranulin.[23,24] It has recently been shown that monocyte p14 (calgranulin B) is phosphorylated in a calcium-dependent manner on the THR residue at position 113.[25] Interestingly, the calgranulin complex specifically inhibits the activity of casein kinase I and II[26] which are threonine kinases.[27] The interrelationship of these enzymes and their inhibitor, calgranulin, which is itself a potential substrate for the kinase activity, needs investigation. Casein kinase II phosphorylates myosin light chain[28] and thus may enhance cell motility. Inhibition of the kinase may account for the inhibitory effect on leucocyte migration of calgranulin.[6] The function of calgranulin in keratinocytes, its localization in a cytoskeletal/junctional plaque and its inter-relationship with protein kinases and their substrates are worthy of further investigation. Experiments are now underway to establish the identity of molecules in cytoskeletal extracts to which calgranulins bind in gel-overlay techniques using recombinant calgranulin protein.

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References

1 Gabrielsen T, Dale I, Brandtzaeg P *et al.* Epidermal and dermal distribution of a myelomonocytic antigen (L1) shared by epithelial cells in various skin diseases. *J Am Acad Dermatol* 1986; **15:** 173–99.

2 Kelly SE, Jones DB, Fleming S. Calgranulin expression in inflammatory dermatoses. *J Pathol* 1989; **159:** 17–21.

3 Kirkham N, Peacock SJ, Jones DB. Monoclonal antibody MAC 387 recognizes a myelomonocytic antigen shared by epithelial cells in inflammatory skin diseases. *Br J Dermatol* 1990; **122:** 61–70.

4 Wilkinson MM, Busuttil A, Hayward C *et al.* Expression pattern of two related cystic fibrosis associated calcium binding proteins in normal and abnormal tissues. *J Cell Sci* 1988; **19:** 221–30.

5 Brandtzaeg P, Jones DB, Flavell DJ, Fagerhol MK. A monoclonal antibody to human monocytes that reacts with the formalin resistant myelomonocytic L1 antigen. *J Clin Pathol* 1988; **41:** 963–70.

6 Zwadelo G, Bruggen J, Gerhards G *et al.* Two calcium binding proteins associated with specific stages of myeloid cell differentiation are expressed by subsets of macrophages in inflammatory tissues. *Clin Exp Immunol.* 1988; **72:** 510–15.

7 Lagasse E, Clerc RG. Cloning and expression of two human genes encoding calcium binding proteins that are regulated during myeloid differentiation. *Mol Cell Biol* 1988; **8:** 2402–10.

8 Gerke V, Weber K. The regulatory chain in the p36-KD substrate complex of viral tyrosine-specific protein kinases is related in

sequence to the S-100 protein of glial cells. *EMBO J* 1985; **4:** 2917–20.

9 Osborn M, Johnsson N, Wehland J, Weber K. The submembranous location of p11 and its interaction with the p36 substrate of pp60 src kinase *in situ*. *Exp Cell Res* 1988; **175:** 81–96.

10 Bikle A, Munson S, Christakos S *et al.* Calmodulin binding to the intestinal brush border membrane: comparison to other calcium binding proteins. *Biochem Biophys Acta* 1989; **1010:** 122–7.

11 Clark BR, Kelly SE, Fleming S. Calgranulin expression and association with the keratinocyte cytoskeleton. *J Pathol* 1990; **160:** 25–30.

12 Shotton DM. Confocal scanning optical microscopy and its applications for biological specimens. *J Cell Sci* 1989; **94:** 175–206.

13 Fleming S, Kelly SE. Confocal laser scanning microscopy: bullous disease. *Am J Dermatopathol* 1990; (in press).

14 Lowrey AA, Kaufman SJ. Membrane-cytoskeleton associations during myogenesis deviate from traditional definitions. *Exp Cell Res* 1989; **183:** 1–23.

15 Garrod DR. Desmosomes, cell adhesion molecules and the adhesive properties of cells in tissues. *J Cell Sci* 1986; **4** (Suppl.): 221–37.

16 Brody I. The ultrastructure of the epidermis in psoriasis vulgaris as revealed by electron microscopy *J Ultrastruct Res* 1962; **6:** 304–67.

17 Pitehka DR, Taggart BN, Hamameto ST. Effects of extracellular calcium depletion on membrane topography and occluding junctions of mammary epithelial cells in culture. *J Cell Biol* 1983; **96:** 613–24.

18 Vilela MJ, Parrish EP, Wright DH, Garrod DR. Monoclonal antibody to desmosomal glycoprotein 1—a new epithelial marker for diagnostic pathology. *J Pathol* 1987; **153:** 365–76.

19 Garrod DR, Fleming S. Early expression of desmosomal components during kidney tubule morphogenesis in human and murine embryos. *Development* 1990; **108:** 313–22.

20 Gentry LE, Rohrschneider LR. Common features of the yes and src gene products defined by peptide specific antibodies. *J Virol* 1984; **51:** 539–46.

21 Burridge K, Fath K, Kelly T *et al.* Focal adhesions: Transmembrane junctions between the extracellular matrix and the cytoskeleton. *Ann Rev Cell Biol* 1988; **4:** 487–26

22 Crompton MR, Moss SE, Crumpton MJ. Diversity in the lipocortin/calpactin family. *Cell* 1988; **55:** 1–3.

23 Glenney JR, Boudreau M, Galgean R *et al.* Association of the S-100 related calpactin light chain with the NH_2-terminal of the 36 kDa heavy chain. *J Biol Chem* 1986; **261:** 10485–8.

24 Dorin JR, Novak M, Hill RE *et al.* A clue to the basic defect in cystic fibrosis from the cloning of the CF antigen gene. *Nature* 1987; **326:** 614–17.

25 Edgeworth J, Freemont P, Hogg N. Ionomycin-regulated phosphorylation of the myeloid calcium-binding protein p14. *Nature* 1989; **342:** 189–92.

26 Murao S, Collart FR, Huberman E. A protein containing the cystic fibrosis antigen is an inhibitor of protein kinases. *J Biol Chem* 1989; **264:** 8356–60.

27 Hathaway G, Traugh JA. Casein kinases—multipotential protein kinases. *Curr Top Cell Regul* 1982; **21:** 101–27.

28 Kelly PT, Shenolikar S Role of autophosphorylation in regulation of calmodulin-dependent protein kinases. *Methods in Enzymology* 1989; **139:** 690–174.

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Human skin basement membrane-associated heparan sulphate proteoglycan: distinctive differences in ultrastructural localization as a function of developmental age

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Summary

Recent studies have demonstrated that skin basement membrane components are expressed within the dermo-epidermal junction in an orderly sequence during human foetal development. We have investigated the ultrastructural localization of basement membrane-related antigens in human foetal skin at different developmental ages using two monoclonal antibodies to a well-characterized basement membrane-associated heparan sulphate proteoglycan. A series of foetal skin specimens (range, 54–142 gestational days) were examined using an immunoperoxidase immunoelectron microscopic technique. In specimens representing very early developmental ages, very diffuse immunoreaction products were detected. However, by approximately 76 gestational days, some accentuation of heparan sulphate proteoglycan was noted along the lamina densa, and by 142 gestational days, the distribution of heparan sulphate proteoglycan was identical to that observed in neonatal and adult human skin. These findings demonstrate that active remodelling of the dermo-epidermal junction occurs during at least the first two trimesters, and affects not only basement membrane-associated structures but also specific antigens.

Basement membranes (BM) are highly organized extracellular matrices which serve a variety of critical functions, depending on which organ and specific basement membrane is considered.[1–6] Although some organ and species specificities exist with regard to the antigenic composition of vertebrate BM, in general each is composed of proteoglycans, glycoproteins, and both collagenous and non-collagenous proteins, which are distributed in an orderly fashion within specific ultrastructural regions. In addition, each known BM component develops in a predictable temporal sequence during foetal development, suggesting the possibility of differences in function during different stages of organogenesis. In skin, for example, it has been shown at the light microscopic level that the more widely distributed BM components, such as laminin, type IV collagen, heparan sulphate proteoglycan, chondroitin 6-sulphate proteoglycan, fibronectin and LDA-1, are present when an intact lamina densa is first observed, whereas other antigens having more restricted localization (i.e., confined to the dermo-epidermal junction) appear later.[7–14] To date, however, it is not known whether temporally dependent ultrastructural differences exist in the distribution of one or more of these BM components.

One of the best characterized ubiquitous BM components is heparan sulphate proteoglycan (HSPG). Recent immunoelectron microscopic studies have demonstrated that a large HSPG, having a core protein of >400 kDa and composed of approximately 30% carbohydrate, is present primarily within the lamina densa and sub-lamina densa regions of the dermo-epidermal junction of neonatal foreskin and adult human skin, and that at the light microscopic level is detectable in human foetal skin as early as 54 gestational days.[14] In the present study, we have sought to determine whether at the ultrastructural level the location of this particular HSPG within foetal skin BM differs as a function of developmental age.

Methods

Source of immunoreagents

Horseradish peroxidase-conjugated goat anti-rat IgG

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and normal goat serum were obtained from Sigma (St Louis, MO, U.S.A.) and Cappel Laboratories (West Chester, PA, U.S.A.), respectively. Unless otherwise specified, all dilutions were prepared in 0·0067 mol/l phosphate-buffered saline (PBS) pH 7·4.

Source of anti-HSPG monoclonal antibodies

The two anti-HSPG monoclonal antibodies used in this study were provided by Dr Alexander V. Ljubimov (All-Union Cancer Research Center of the U.S.S.R. Academy of Medical Sciences, Moscow, U.S.S.R.). The parent monoclonal antibody-producing rat x mouse hybridoma cell lines, C11L1 and A7L6, were generated following immunization of Fisher rats with a murine EHS tumour laminin preparation containing entactin and HSPG.[15,16] Both of these IgG2a, κ monoclonal antibodies, obtained from hybridoma culture supernatants, have been shown to have binding specificity for the core protein of a high molecular weight (>400 kDa), low buoyant density HSPG present in EHS tumour and cultured human endothelium.[17]

Source of tissues

Sixteen skin specimens from aborted normal human foetuses ranging in gestational age from 54 to 142 days were provided by Dr Karen Holbrook and the Central Laboratory for Human Embryology at the University of Washington (Seattle, WA, U.S.A.) These foetal skin specimens were previously shown to express laminin, type IV collagen, LDA-1 (a novel ubiquitous collagenase-resistant lamina densa antigen), fibronectin and chondroitin 6-sulphate containing proteoglycan over this entire range of gestational ages.[7,11,12]

Indirect immunoelectron microscopy

Indirect immunoelectron microscopy was performed with each anti-HSPG core protein monoclonal antibody, using 6–8-μm-thick cryosections of unfixed cryopreserved human foetal skin specimens as tissue substrates. Tissue sections were serially incubated with normal goat serum (1:10; 20 min; room temperature), anti-HSPG core protein monoclonal antibody (1:10; 18 h; 4°C), and horseradish peroxidase-conjugated goat anti-rat IgG (1:20; 18 h; 4°C). As a negative control, normal rat serum (1:10) was substituted for each of the two monoclonal antibodies. After re-fixation with 1% glutaraldehyde, tissue sections were then incubated with diaminobenzidine and hydrogen peroxide, post-fixed with 1% osmium tetroxide, dehydrated with a gradient alcohol series, and embedded in Spurr's resin. Ultrathin sections were counterstained with lead citrate and then examined under a Phillips 300 electron microscope.

Results

The ultrastructural localization for each of the two anti-HSPG core protein monoclonal antibodies was identical when compared on matched foetal skin samples of the same gestational age. Representative findings are illustrated in Figure 1. At approximately 54 gestational days (Fig. 1a), a time closely corresponding to that in which the lamina densa is first detectable along the dermo-epidermal junction,[18] immunoreaction products were diffusely present in amorphous band-like array throughout the entire region of the BM, extending from just beneath the basilar keratinocyte cytoplasmic membrane into the upper portion of the papillary dermis. Similarly, diffuse staining was noted at 67 gestational days (Fig. 1b). By 76 days (Fig. 1c) and in all later aged tissues, however, increasingly dense, linear immunoreactant deposition could be noted, with primary accentuation along the lamina densa. By 142 gestational days (Fig. 1f), the distribution of HSPG core protein was found to be identical to that previously observed in human neonatal foreskin and adult skin.[14] In particular, whereas the primary localization of HSPG core protein was readily detectable in a defined linear array along the lamina densa, specific immunoreaction products could also be observed around individual collagen fibrils and some anchoring fibrils in the uppermost part of the papillary dermis, as well as more weakly staining focal portions of the lamina lucida directly underneath basilar keratinocyte-associated hemidesmosomes. It could not be determined whether some of these immunoreactants were present within the hemidesmosomes themselves, due to technical limitation in both resolution of epidermal cellular ultrastructural morphology (due to cryopreservation-induced artefacts which were present within the keratinocytes) and the staining technique employed.

Discussion

Electron microscopic studies have demonstrated that the human dermo-epidermal junction is composed of a variety of structures that include the lamina lucida, lamina densa, hemidesmosome, anchoring filament, sub-basal dense plate, and anchoring fibril. The appearance of each of these structures occurs in a predictable sequence during the first and second trimesters.[4–6,18–23]

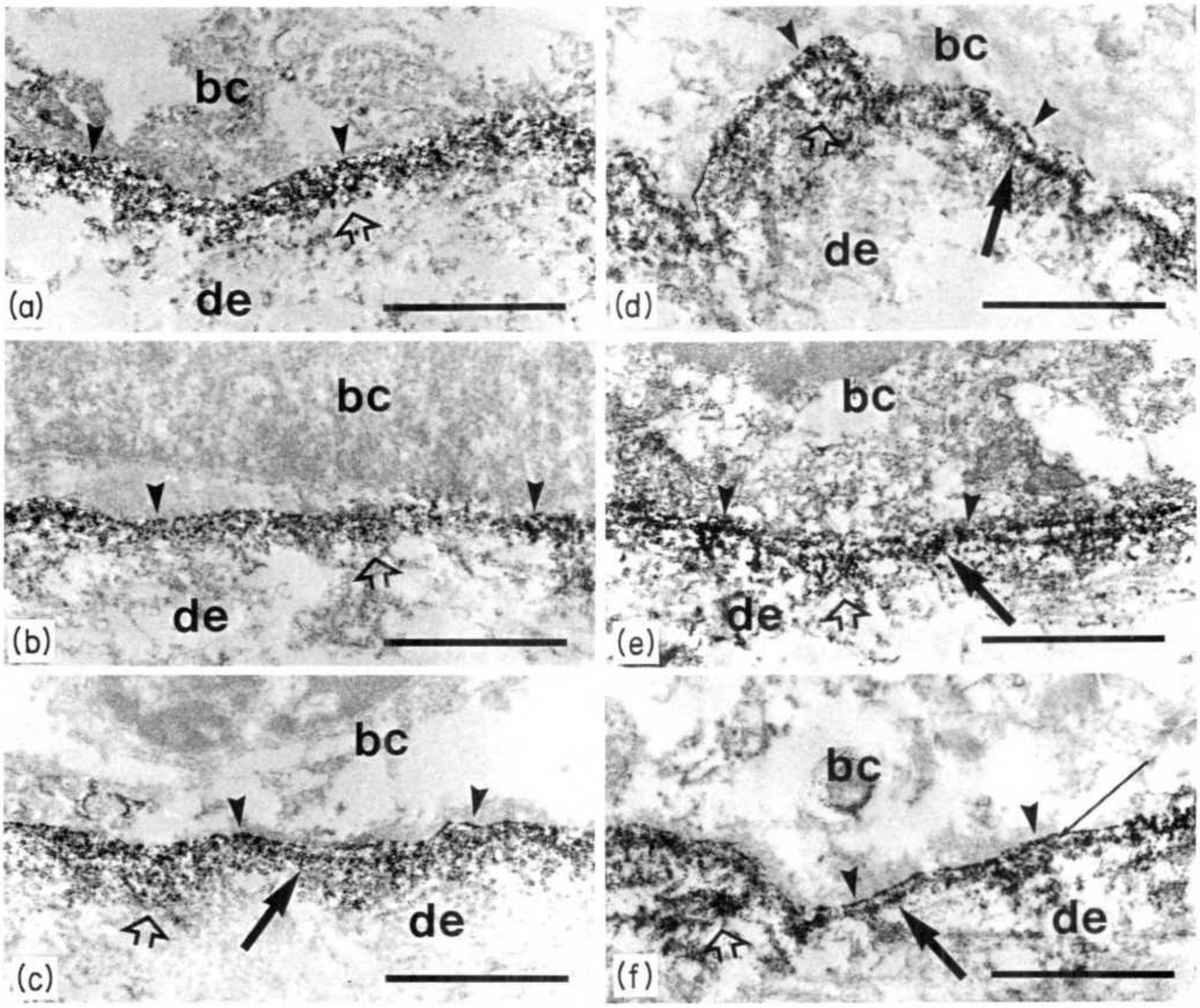

Figure 1. Indirect immunoelectron microscopic study of human foetal skin, using a monoclonal antibody specific for heparan sulphate proteoglycan core protein and an immunoperoxidase technique. Figures a–f correspond to 54, 67, 76, 81, 86 and 142 gestational days, respectively. bc, basal cell (keratinocyte); de, dermis. All magnification bars = 1 μm; magnifications × 25,800. (*a*) and (*b*) Immunoreaction products (open arrow) are diffusely distributed in a broad, band-like array along the dermo-epidermal junction, without accentuation of any specific ultrastructural regions. Arrowheads denote approximate location of basilar keratinocyte cytoplasmic membrane. (*c*) By approximately 76 gestational days, immunoreaction products begin to become somewhat more confined, in linear array, to the lamina densa (thick arrow) and to a lesser extent, to the uppermost papillary dermis (open arrow). Arrowheads denote location of developing hemidesmosomes along the basilar keratinocyte cytoplasmic membrane. (*d*) and (*e*) By 81 and 86 gestational days, respectively, the primary localization of immunoreactants is along the lamina densa (thick arrow) although lesser amounts are still detectable within the upper dermis (empty arrow). (*f*) By 142 gestational days, virtually all of the immunoreactants are confined in almost continuous array to the lamina densa (thick arrow). Whereas more diffuse staining of the upper dermis is no longer noted, focal deposits of immunoreactants may still be seen around isolated upper dermal collagen fibres (open arrow) and anchoring fibrils, as well as focally and weakly within the lamina lucida (thin arrow), especially in areas directly underneath hemidesmosomes (arrowheads).

With the exception of a recently published study on the ultrastructural detection of type VII collagen in early foetal skin specimens,[24] all previous work on the antigenicity of foetal skin BM has been confined to immunofluorescence studies. From these it is clear that there is a temporal sequence for the expression of many of these antigens.[7-14] In the present study, we have used immunoelectron microscopy to investigate the ultrastructural localization of one such ubiquitous BM component, heparan sulphate proteoglycan core protein.

As previously described, a rather amorphous distribution of HSPG was noted along the dermo-epidermal junction in the earliest age of the human foetal skin specimens that were examined. With increasing developmental age, however, the distribution of HSPG progressively approached that observed in neonatal foreskin and adult human skin,[14] including around other structures (i.e., collagen fibres, anchoring fibrils) closely associated with the dermo-epidermal junction. These findings demonstrate that human foetal BM is subjected to a dynamic process of antigenic remodelling and re-distribution throughout the course of development, rather than simply acquiring additional morphological structures, such as hemidesmosomes and anchoring fibrils, with increasing age. A previous study of fibronectin distribution in developing rat skin has shown some similarity to these findings.[25] Whereas in the early embryonic rat dermo-epidermal junction fibronectin was diffusely distributed, a more restricted pattern of fibronectin staining (i.e., to the lamina lucida and sublamina densa regions) was apparent as epidermal stratification proceeded at about 17 gestational days. Unlike the findings in the present study with HSPG, however, a marked decrease in the amount of fibronectin staining was seen during subsequent stages of rat skin development.[25]

It is unknown whether, or how, such antigenic redistribution might influence the timing of or sites for the appearance of other BM-associated structures. Similarly, it is impossible, based upon the approach taken in this particular study, to identify a single specific developmental time or structure which signals a more adult-like redistribution of HSPG along the dermo-epidermal junction, although the detection of some initial accentuation of HSPG along the lamina densa by approximately 10 gestational weeks might suggest some rough correlation with the time at which hemidesmosomes are reportedly first observed.[19,23] However, these data do suggest that other ubiquitous BM components may experience similar ultrastructural re-distribution in the human foetus, with their final localization probably reflecting a compromise between those spatial orientations most optimal for interactions among specific BM molecules and the inclusion of specific structures within the maturing basement membrane zone. In support of the latter hypothesis, preliminary data from our laboratories with monoclonal antibodies to the A and B chains of human laminin demonstrate a similar tendency for an amorphous distribution along the dermo-epidermal junction during early foetal skin development, and more restricted distribution with advancing gestational age.[26] As yet there is no direct way to determine whether these temporal differences in the ultrastructural location of HSPG may also indicate changes in function as a reflection of gestational age.

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References

1 Bernfield M, Banerjee SD, Koda JE, Rapraeger AC. Remodelling of basement membrane as a mechanism of morphogenetic tissue interaction. In: *The Role of Extracellular Matrix in Development* (Trelstad RC, ed), New York: Alan R. Liss, 1987, 545–72.

2 Bissell MJ, Hall HG, Parry G. How does the extracellular matrix direct gene expression? *J Theor Biol* 1982; **99**: 31–68.

3 Hay ED. Cell-matrix interaction in the embryo: cell shape, cell surface, cell skeletons, and their role in differentiation. In: *The Role of the Extracellular Matrix in Development* (Trelstad RC, ed), New York: Alan R. Liss, 1984; 1–31.

4 Briggaman RA. The epidermal–dermal junction and genetic disorders of this area. In: *Biochemistry and Physiology of the Skin* (Goldsmith LA, ed), New York: Oxford University Press, 1983; 1001–24.

5 Fine J-D. The skin basement membrane zone. In: *Advances in Dermatology* (Callen J, ed), 2nd edn. Chicago: Year Book Publishers, 1986; 283–303.

6 Katz SI. The epidermal basement membrane zone—structure, ontogeny, and role in disease. *J Am Acad Dermatol* 1984, **11**: 1025–37.

7 Fine J-D, Smith LT, Holbrook KA, Katz SI. The appearance of four basement membrane zone antigens in developing human fetal skin. *J Invest Dermatol* 1984; **83**: 66–69.

8 Lane AT, Helm KF, Goldsmith LA Identification of bullous pemphigoid, pemphigus, laminin, and anchoring fibril antigens in human fetal skin. *J Invest Dermatol* 1985; **84**: 27–30.

9 Muller HK, Kalnins R, Sutherland RC. Ontogeny of pemphigus and bullous pemphigoid antigens in human skin. *Br J Dermatol* 1973; **88**: 443–6.

10 Paller AS, Queen LL, Woodley DT *et al.* Organ-specific, phylogenetic, and ontogenetic distribution of the epidermolysis bullosa acquisita antigen *J Invest Dermatol* 1986; **86**: 376–9.

11 Fine J-D, Gay S. LDA-1: A ubiquitous non-collagenous lamina densa component of basement membrane detected by monoclonal antibody technique. *J Invest Dermatol* 1986; **86**: 286–9.

12 Fine J-D, Couchman JR. Chondroitin-6-sulfate-containing proteoglycan: a new component of human skin dermoepidermal junction. *J Invest Dermatol* 1988; **90**: 283–8.

13 Fine J-D, Horiguchi Y, Jester J, Couchman JR. Detection and partial characterization of a mid-lamina lucida–hemidesmosome-associated antigen (19-DEJ-1) present within human skin. *J Invest Dermatol* 1989; **92**: 825–30.

14 Horiguchi Y, Couchman JR, Ljubimov AV *et al.* Distribution, ultrastructural localization, and ontogeny of the core protein of a heparan sulfate proteoglycan in human skin and other basement membranes. *J Histochem Cytochem* 1989; **37**: 961–70.

15 Fujiwara S, Wiedemann H, Timpl R *et al.* Structure and interactions of heparan sulfate proteoglycans from a mouse tumor basement membrane *Eur J Biochem* 1984; **143**: 145–57

16 Ljubimov AV, Afanasjeva AV, Litvinova LV, Senin VM. Basement

membrane components produced by a mouse ascites teratocarcinoma TB24. *Exp Cell Res* 1986; **165:** 530–40.

17 Couchman JR, Ljubimov AV. Mammalian tissue distribution of a large heparan sulfate proteoglycan detected by monoclonal antibodies. *Matrix* 1990; **9:** 311–21.

18 Breathnach AS, Robins J. Ultrastructural feature of epidermis of a 14 mm (6 weeks) human embryo. *Br J Dermatol* 1969; **81:** 504–16.

19 Holbrook KA. Structure and function of the developing human skin. In: *Biochemistry and Physiology of the Skin* (Goldsmith LA, ed), New York: Oxford University Press, 1983: 64–101.

20 Kobayasi T. An electron microscope study on the dermo-epidermal junction. *Acta Derm Venereol (Stockh)* 1961; **41:** 481–91.

21 Palade G, Farquhar M. A special fibril of the dermis. *J Cell Biol* 1965: **27:** 215–24.

22 Tidman MJ, Eady RAJ. Ultrastructural morphometry of normal human dermal-epidermal junction: The influence of age, sex, and body region on laminar and nonlaminar components. *J Invest Dermatol* 1984; **83:** 448–53.

23 Holbrook KA, Smith LT. Ultrastructural aspects of human skin during embryonic, fetal, neonatal and adult periods of life. In: *Morphogenesis and Malformations of the Skin* (Blandau RJ, ed), XVII (no. 2). New York: Alan R. Liss, 1981; 9–38.

24 Smith LT, Sakai LY, Burgeson RE, Holbrook KA. Ontogeny of structural components at the dermal-epidermal junction in human embryonic and fetal skin: the appearance of anchoring fibrils and type VII collagen. *J Invest Dermatol* 1988; **90:** 480–5.

25 Gibson WT, Couchman JR, Weaver AC. Fibronectin distribution during the development of fetal rat skin. *J Invest Dermatol* 1983; **81:** 480–5.

26 Horiguchi Y, Fine J-D, Couchman JR, Engvall E. Expression of heparan sulfate proteoglycan and laminin in developing human fetal skin basement membrane: differences in ultrastructural localization are dependent on developmental age. *J Invest Dermatol* 1990; **94:** 536.

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Hair growth inhibition by heparin in mice: a model system for studying the modulation of epithelial cell growth by glycosaminoglycans?

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Summary

Although it is known that glycosaminoglycans (GAG) can affect hair growth, their role in follicular growth regulation is not yet understood. We have administered one such GAG, heparin, to anagen-induced mice to help to elucidate this role, using the C57 Bl-6 model for murine hair growth studies. Heparin was found to exert dose- and hair cycle-dependent, differential effects on skin epithelial cell functions. Intraperitoneal, but not topical application of heparin inhibited the development of anagen follicles in anagen-induced mice as assessed by morphometry. When the skin of heparin-treated mice was cultured in an organ culture assay, the epidermis showed a significant increase in the synthesis of arginine-rich proteins (ARP), while epithelial bulb, but not epidermal cell proliferation was reduced in comparison with control skin. In mouse-skin organ culture, the effects of direct administration of heparin to the medium on epithelial cell proliferation and ARP synthesis were dose-dependent and varied (inhibition or stimulation of either parameter), depending on the stage of the hair cycle, the cell population in question (epidermal vs. epithelial bulb keratinocytes), and the length of incubation. PAM cell and mouse dermal papilla cell proliferation *in vitro* was inhibited by heparin. We conclude that heparin may be a useful tool for characterizing the role of GAG in epithelial cell biology and epithelial–mesenchymal interactions in general, and in hair growth in particular.

Hair growth results from epithelial–mesenchymal interaction[1] and because programmed changes in the extracellular matrix (ECM) composition are an important mode of intercellular communication,[2–4] the role that ECM plays in this interaction is of interest.[5] The ECM consists primarily of fibrous proteins embedded in a hydrated polysaccharide gel formed by glycosaminoglycans (GAG), which are commonly linked to protein in the form of proteoglycans.[2] Due to their capacity for multiple interactions with other molecules and their role in cell adhesion and cell–cell interactions as well as in the control of cell proliferation and morphogenesis they have been viewed as a multipurpose 'glue' in cellular interactions.[4,6,7] In the context of hair growth, glycosaminoglycans (GAG)—particularly heparin and heparan sulphate, two polysaccharides abundant in skin[2,3]—are of special interest, because a number of clinical and experimental observations suggest an important role for GAG in the regulation of hair growth.

It has long been recognized that reversible, dose-dependent hair loss is one of the most common side-effects of systemic heparin therapy in man, reportedly occurring in up to 50% of patients.[8–10] GAG storage diseases with abnormal dermal accumulation of GAG (e.g. mucopolysaccharidoses, myxoedema, cutaneous mucinoses) are frequently associated with changes in hair growth; dependent on the type of GAG accumulated, this results in either follicular atrophy or focal hypertrichosis.[8,11] That GAGs can affect hair growth is also suggested by alopecia mucinosa, a rare disorder of follicular GAG production, which affects the pilosebaceous apparatus and results in hair loss.[12,13]

Evidence has accumulated that the hair cycle is associated with significant, cycle stage-specific and tightly regulated changes in the GAG composition and concentration in the skin, which are particularly evident in rodents.[14–18] The heparin concentration in rat skin, for example, shows a sharp, short-lasting peak in anagen, with baseline levels in catagen and telogen; correspondingly, the number of dermal mast cells (main producers of and cellular reservoir for heparin) changes during the different phases of the rat hair cycle.[19] The rat hair cycle also exhibits considerable cycle-associated

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fluctuations of skin proteoglycans.[16,18] Intradermal GAG-injections were reported to stimulate hair growth in rabbits,[20] while a host of growth-modulatory effects of different GAGs on epithelial and mesenchymal cells *in vitro* have been reported.[21–24]

How heparin or other GAGs affect hair growth is as yet unclear as it has not been studied systematically. Understanding these hair growth-inhibitory effects of heparin is not only clinically important, but may also provide new insight into the biological functions of heparin and other GAGs in general in epithelial tissue growth and epithelial–mesenchymal interactions. In addition to the anti-coagulative properties of heparin, a wide range of reported heparin effects must be considered such as antimitotic effects, cytokine-binding and immunomodulation *in vitro*; antiangiogenic, tumour growth-inhibiting and morphogenic effects *in vivo*.[7,22,24–26]

Using the previously described C57 Bl-6 mouse model for hair growth studies,[27–30] heparin was administered to anagen-induced mice *in vivo*, and to anagen mouse skin grown in organ culture. The results of this pilot study suggest that heparin modulates skin epithelial cell growth in a hair cycle-dependent fashion, has differential effects on epidermal and epithelial bulb keratinocyte growth *in vivo* as well as *in vitro*, and can stimulate the synthesis of arginine-rich proteins by keratinocytes (as a by-product, this study also reports the pattern of arginine-rich protein synthesis in murine skin during a full hair cycle).

Methods

Animals, cells and materials

C57 Bl-6 mice (syngeneic, female, 6–8 weeks; Charles River, Kingston, NY, U.S.A.) in the telogen stage of the hair cycle (pink back skin) were anagen-induced by wax epilation as previously described.[28–30] Mouse dermal papilla cells were cultured from papillae that had been microdissected from the anagen vibrissae follicles of C57 Bl-6 mice using the method of Messenger[31] as modified by Link *et al.*[5] and were cultured in 199 E medium with 10% FBS and antibiotics and used at the second passage. PAM cells (a mouse keratinocyte cell line) were a gift from Dr R.Langdon, and were grown in DME with 10% FBS and antibiotics. Media were obtained from the Yale Media Facility, tissue culture reagents and FBS from Gibco, radioisotopes ([^{3}H]-thymidine, [^{3}H]-arginine, [^{3}H]-leucine) from Amersham. Heparin–sodium salt (porcine intestinal mucosa, #H-3125), was from Sigma and was diluted in Tyrodes' balanced salt solution. Heparin gel ('Thrombocutan-Ultra Gel', containing 500 I.U. heparin–sodium/g) was a gift from Dorsch Co., Munich, Germany.

Heparin in vivo

One hour after anagen induction,[28–30] heparin was administered intraperitoneally or topically to mice for 5 days and mouse tissue harvested for histology or for skin organ culture. Intraperitoneally, 0·5 ml of 0–10 μg/ml heparin in Tyrodes' were administered once daily, while topically, either 100 μg/ml heparin in 80% propylene glycol, 10% ethanol and 10% water or heparin gel (Dorsch) were applied twice daily (08–10.00, 16–18.00) with a brush to the back of anagen-induced mice. The effect of *in vivo* heparin administration on anagen follicle development was assessed histologically and quantified by morphometry as previously described.[27–29]

The skin thickness, which greatly increases during anagen development,[28,32,33] was measured semi-blind between the basement membrane and the upper margin of the panniculus carnosus as a reliable, indirect quantitative parameter for anagen development. This correlated predictably with the development of spontaneous as well as traumatically induced anagen and excluded the initial epidermal wounding response to the plucking stimulus.[27–29,34,35] The appearance of trichohyaline granules (THG) was assessed semi-blind as a specific, qualitative marker for anagen follicles (THG is only present in the internal root sheath of anagen follicles[29,36,37]) and expressed as number of microscopic fields positive for THG per six fields (4 × 6 fields counted, × 100 magnification). The macroscopic skin colour and texture were recorded.

Heparin ex vivo

Using the previously described mouse-skin organ culture assay,[28] back skin from mice treated for 5 days *in vivo* with either 0·5 ml of 0·5 μg/ml heparin or vehicle (as above) was harvested. Skin fragments (4 mm) from nine punch biopsies per mouse and culture dish were cultured at the air–liquid interphase (dermis down on steel grids, in 5 ml DME with 10% FBS and antibiotics) and pulsed with either 5 μCi/ml [^{3}H]-Tdr or 1 μCi/ml [^{3}H-]Arg. After a 24-h incubation ('short-term assay', see below), the tissue fragments were washed in PBS, incubated overnight in 2 M NaBr at 4°C, washed again and the epidermis and dermis separated, and liquid scintillation spectrometry performed on each individual skin compartment as previously described.[28–30,38] Excluding the highest and the lowest count in each group, radioisotope incorporation was expressed as the mean c.p.m. ± 1 SE ($n = 7$ per group).

Heparin in vitro

Using the same skin organ culture assay, heparin (0–5 μg/ml in 50 μl of Tyrodes') was directly administered to the medium (pulsed at 0 h with [^{3}H]-Tdr or [^{3}H]-Arg as above). Back skin fragments were obtained from either telogen or early anagen (day 2 after induction) or late anagen (day 8 after induction) mice. Two protocols were used: 'short-term' assays were done with 4-mm punches, incubated for 24 h, while 'long-term' assays (early anagen only) were done with 2-mm punches, incubated for 48 h and pulsed with heparin for a second time at 24 h. The viability of 2 and 4 mm telogen and anagen skin fragments, cultured under assay conditions for 48 and 24 h, respectively, had previously been assertained, assessing [^{3}H]-Tdr and [^{3}H]-Arg incorporation by autoradiography and scintillation spectrometry (R.Paus, unpublished data). In order to get an indication of heparin's effect on general protein synthesis (preliminary skin organ culture assays with heparin administration had suggested no effect of heparin on [^{3}H]-leucine or [^{35}S]-cysteine incorporation), [^{3}H]-Leu incorporation (1 μCi/ml) into early anagen skin in long-term organ culture (2 mm, 48 h, day 2 skin) was compared with that of [^{3}H]-Arg.

Preliminary experiments had suggested an antimitotic effect of heparin *in vitro* (0·005–500 μg/ml) on rat and mouse papilla cells (MDP), rat dermal fibroblasts, A431 cells and human keratinocytes. Therefore, MDP cells (P-2) were plated in 24-well plates at 50,000 cells/well in 199E medium with 10% FBS and PAM cells plated at 100,000 cells/well in DME with 10% FBS 24 h before the experiment. After exchanging the media (3 ml) and pulsing each well with either 5 μCi/ml [^{3}H]-Tdr or 1 μCi/ml [^{3}H]-Arg, 0–50 μg/ml heparin in Tyrodes' were added. After incubating all groups for 18 h at 37°C, 5% CO_2 air, 100% humidity, all wells were washed four times with cold PBS, the cells lysed in 300 μl of 0·1 M NaOH/0·1% SDS, and the total amount of lysate counted by liquid scintillation spectrometry as before (fourth wash: background control).

Hair cycle-associated ARP synthesis

Because the hair cycle-associated proliferation kinetics of mouse epidermis and epithelial bulbs had previously been characterized by measuring incorporation of [^{3}H]-Tdr into skin fragments from different stages of the hair cycle,[30] we characterized the hair cycle-associated pattern of arginine-rich protein (ARP) synthesis in mouse epidermis and epithelial bulbs, a keratinocyte differentiation marker,[29] by assessing incorporation of [^{3}H]-Arg into skin fragments harvested between days 0 and 20 after anagen induction. Preliminary experiments revealed significant alterations of [^{3}H]-Arg incorporation in skin organ culture by addition of heparin to the medium. Fragments were harvested on the day indicated (Fig. 1), grown in short-term skin organ culture (4 mm, 24 h incubation) as above, and scintillation spectrometry performed on either the whole skin fragment, or the separated epidermal or dermal compartment; [^{3}H]-Arg incorporation occurs primarily in epidermal and epithelial bulb keratinocytes.[29]

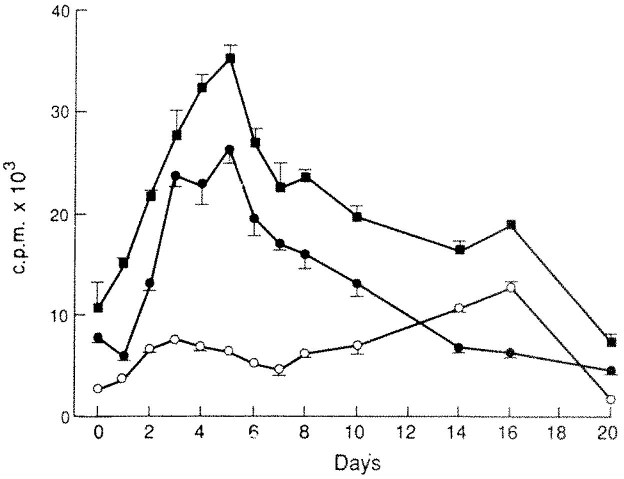

Figure 1. Dynamics of arginine-rich protein (ARP) synthesis during the murine hair cycle. The hair cycle-associated synthesis of ARP was measured by incubating C57 Bl-6 mouse skin fragments from different stages of an induced hair cycle (day 0–20 harvested at the day indicated) in DME + 10% FBS + 1 μCi/ml [^{3}H]-Arg at the air–liquid interface for 24 h, before counting either the whole fragment or epidermal and dermal fragment individually by liquid scintillation spectrometry. Counts are given in c.p.m. ± SE and represent data calculated from 7–21 fragments from 1–3 mice per point. ■, total c.p.m. of the whole skin fragment (epidermis and dermis not separated); ●, c.p.m. of the epidermal fragment; ○, c.p.m. of the dermal fragment (epidermis and dermis separated by NaBr incubation).

Results

Arginine-rich protein synthesis during the mouse hair cycle (ex vivo assay)

The first set of experiments further defined the previously reported mouse-skin organ culture assay.[28–30] The pattern of arginine-rich protein (ARP) synthesis during the mouse hair cycle is shown in Figure 1, which demonstrates that epidermal and dermal fragments exhibited two distinct patterns of Arg-incorporation. While incorporation of Arg into the epidermis increased rapidly after anagen induction in telogen mice and peaked around 6 days after anagen induction, the increase in dermal Arg incorporation was more subtle, less rapid and showed two peaks: one peak around day 3 after anagen induction, and the other towards the end of anagen (day 16), shortly before the onset of catagen (day 17–20).

Heparin administration in vivo (morphometric and ex-vivo assays)

Intraperitoneal administration of heparin to anagen-induced mice once daily over 5 days resulted in a significant retardation of anagen follicle development that was evident both macroscopically and microscopically. The inhibition of anagen development by heparin was dose-dependent and maximal at a medium-range concentration (Table 1) using the microscopic measurement of skin thickness as an indirect, quantitative parameter and the measurement of trichohyalin granule-formation as a direct, qualitative parameter for anagen development.[27,28]

Mice injected intraperitoneally with 0·25 μg heparin/day showed a skin thickness of 230±3(SE) μm and significant retardation of trichohyalin granule development as compared to controls (skin thickness: 250 μm±5). At this maximum inhibitory concentration, some skin sections showed epidermal hyperplasia with ortho- and parakeratosis as well as isolated mitotic figures in the basal keratinocyte layer of the epidermis. None of these changes were seen in control skin. No morphological abnormalities were seen in the hair follicles of either test or control mice. Macroscopically, the heparin-injected mice had a pink skin colour compared to the grey colour of control mice, reflecting the retardation of anagen-associated melanogenesis[38] by heparin, and showed variable degrees of epidermal scaling. When the experiment was repeated with different batches of C57 Bl-6 mice, the inhibitory effect of intraperitoneal heparin administration on morphometrically assessed anagen development was even more pronounced (Table 1, Experiment B shows one example).

This effect was quantifiable by measurement of radioisotope incorporation (*ex-vivo* assay), when mouse skin from animals treated daily for 5 days with 0·25 μg heparin or vehicle alone was cultured at the air–liquid interphase (Table 2). The dermal [^{3}H]-Tdr incorporation (reflecting epithelial bulb keratinocyte proliferation) of

Table 1. Effects of heparin administration *in vivo* on anagen development. Heparin was administered intraperitoneally (Exp. A) once daily over 5 days to anagen-induced mice (0·5 ml of conc. shown below; vehicle: Tyrodes'). Parameter: microscopic measurement of differences in skin thickness and of the appearance of trichohyalin granules (THG); number of fields positive for THG per six fields. Exp. B shows a representative repeat of experiment A. Topical heparin administration was done by applying twice daily to the back of anagen-induced mice for 5 days, (control: vehicle only) either 100 μg/ml heparin in 80% propylene glycol, 10% ethanol, 10% H_2O, (Exp. C) or heparin-gel (Thrombocutan-Ultra Gel, 500 I.E. heparin-sodium/g) (Exp. D)

Experiment	*n*(mice)	Skin thickness (μm±SE)		THG (±SE)
Intraperitoneal heparin				
A				
Control	4	250(5)		5·3(0·3)
Hep 10 μg/ml	6	240(4)		3·2(0·3)
Hep 0·5*	10	230(3)	[*P*<0·01]	2·0(0·3)
Hep 0·005	8	260(5)		4·3(0 3)
B				
Control	5	265(6)		5·5(0·3)
Hep 0·5 μg/ml*	5	210(4)	[*P*<0·001]	1·8(0·4)
Topical heparin administration				
C				
Heparin in PG/ethanol				
THG (fields pos/6 fields):		Hep 4·6±2·4(SD)		*n*=5
		co. 5.9±0·2(SD)		*n*=5
Macroscopic: no difference in skin colour or texture between exp. and co.				
D				
Heparin gel				
THG:		Hep. 5·1±0·5(SD)		*n*=5
		co. 5·7±0·3(SD)		*n*=5
Macroscopic: no difference in skin colour or texture between exp. and co.				

* Histologically, epidermis thickened, ortho- and parakeratotic epidermal hyperplasia, single mitotic figures; macroscopically, skin pink instead of grey (controls), and palpably thinner than controls; epidermal scaling

Table 2. Heparin in *ex vivo* skin organ culture. *In vivo* administration of 0·5 μg/ml heparin or vehicle control as in Table 1 (experiment A) over 5 days, then skin organ culture (4-mm fragments, 24 h incubation with [^{3}H]-Tdr, [^{3}H]-Arg; *n*=4 mice/group, 4×7 fragments. c.p.m.±SE)

	[^{3}H]-Tdr	*P*	[^{3}H]-Arg	*P*
Epidermis				
Control	33755±4754(SE)		16089±1697	
Heparin	39767±2669	<0·05	27174±1352	<0·001
Dermis				
Control	15474±1346		5969±339	
Heparin	10209±427	<0·05	5638±365	>0·05

test skin was significantly lower than that of control skin; epidermal Tdr incorporation of test skin, in contrast, was slightly higher than that of control skin (though statistically not significant). Unexpectedly the test epidermis showed significantly more [^{3}H]-Arg incorporation than the control epidermis, while no differences were found in the dermal Arg-incorporation.

No significant inhibition of anagen development could be achieved by topical administration of heparin in the two topical formulations chosen (no difference in skin colour between test and control animals). After applying 100 μg/ml heparin in 80% propylene glycol, 10% ethanol and 10% water or vehicle alone twice daily to anagen-induced mice for 5 days, skin sections of control animals had, out of six microscopic fields, 5·9 ± 0·2 (SD) positive for trichohyaline granules, while the score in test animals was 4·6 ± 2·4 (SD) fields. When a commercially available heparin gel, used for topical heparin administration in humans ('Thrombocutan Ultra Gel', 500 I.E. heparin–sodium/g), was applied, the respective values were 5·7 ± 0·3 for control, and 5·1 ± 0·5 for test animals.

Heparin administration in vitro (skin organ culture and cell culture)

When heparin was directly administered to long- or short-term mouse-skin organ culture, there were marked differences in the effects of heparin application on [^{3}H]-Tdr and [^{3}H]-Arg incorporation (Table 3). The effects of heparin on both Arg and Tdr incorporation were dose-dependent, and dependent on the stage of the hair cycle of the skin harvested for organ culture, the localization of epithelial cells (epidermal vs. epithelial bulb) as well as on the length of incubation.

In long-term culture of early anagen skin (day 2; 48 h incubation, 2-mm punches), epidermal Tdr incorporation was significantly stimulated by heparin administration, while dermal Tdr incorporation was inhibited (Table 3). Heparin administration also markedly stimulated the incorporation of Arg into the epidermis as compared to controls, while that of the dermis was less markedly increased. Such a stimulation of ARP synthesis by heparin has not been reported before. Leucine incorporation as a parameter for general protein synthesis, in contrast, was unaffected by heparin application *in vitro* (0·5 μg/ml) in either epidermis or dermis.

However, in short-term organ culture of early anagen skin (day 2; 24 h, 4-mm punches) heparin administration significantly inhibited epidermal and dermal Tdr incorporation; epidermal Arg incorporation was slightly

Table 3. Heparin *in vitro:* skin organ culture. Heparin in the concentrations listed (0–5 μg/ml in 50 μl of vehicle = Tyrodes') was added to mouse skin fragments grown at the air–liquid interphase in organ culture (DME + 10% FBS, pulsed at 0 h with 5 μCi[^{3}H]-Tdr or 1 μCi[^{3}H]-Arg). Short-term culture was performed with 4-mm fragments, harvested from either telogen, early anagen (day 2 post-anagen-induction), or late anagen skin (day 8 post-anagen induction) (experiments B, C, D), incubated for 24 h and Tdr or Arg incorporation into the NaBr-separated epidermal or the dermal fragment measured by scintillation spectrometry. Long-term culture was performed with 2-mm skin fragments, taken from early anagen skin and incubated for 48 h (A) (viability established, see Methods). Data given: c.p.m. ± SE, $n = 7$ fragments per group (all back skin fragments from one entire experiment taken from the same mouse). The general tendency of results was in agreement with previously performed organ culture series

Experiment	Epidermis	*P*	Dermis	*P*
A Early anagen long-term skin organ culture				
[^{3}H]-Tdr				
Control	12,887(1260)		15,741(1568)	
0·05	22,339(3242)	<0·01	17,092(1060)	>0·05
0·5	22,322(2200)	<0·01	8028(1392)	<0·01
5	31,582(1439)	<0·001	10,644(480)	<0·01
[^{3}H]-Arg				
Control	6925(943)		3977(671)	
0·05	7757(1847)	>0·05	3292(457)	>0·05
0·5	17,291(1247)	<0·001	4494(516)	>0·05
5	25,650(1731)	<0·001	7265(498)	<0·05
[^{3}H]-Leu				
Control	13,280(2115)		4432(681)	
0·5	14,534(1766)	>0·05	5523(622)	>0·05
B Early anagen short-term skin organ culture				
[^{3}H]-Tdr				
Control	42,399(1351)		26,733(2672)	
0·5	15,523(1099)	<0·001	14,888(1041)	<0·01
[^{3}H]-Arg				
Control	10,071(811)		1795(231)	
0·5	8251(741)	>0·05	1428(101)	>0·05
C Telogen short-term skin organ culture				
[^{3}H]-Tdr				
Control	5956(1173)		5531(1138)	
0·5	11,028(1923)	<0·001	10,800(1123)	<0·001
[^{3}H]-Arg				
Control	4461(431)		2313(305)	
0·5	4588(337)	>0·05	1714(113)	<0·05
D Late anagen short-term skin organ culture				
[^{3}H]-Tdr				
Control	10,022(700)		18,122(2072)	
0·5	10,510(782)	>0·05	15,945(2188)	>0·05
[^{3}H]-Arg				
Control	9525(626)		9797(1197)	
0·5	7308(437)	>0·05	7110(1152)	>0·05

Table 4. Heparin *in vitro:* cell culture. Mouse dermal papilla cells (MDP, P-2) were grown in 199E+10% FBS while PAM cells were grown in DME+10% FBS, and plated in 24-well plates ($n=12$ wells per PAM group, $n=9$ wells per MDP group). After media exchange, heparin was added (0–50 μg/ml in 50 μl Tyrodes') together with 5 μCi/ml [^{3}H]-Tdr, and after 18 h incubation the c.p.m. + SE of all washed and lysed cells measured by liquid scintillation spectrometry (background c.p.m. of last wash: 30–62 c.p.m.)

Tdr	MDP	*P*	PAM	*P*
Control	17,848(1356)		21,545(1151)	
50	9358(780)	<0·01	3670(499)	<0·001
5	3446(483)	<0·001	5128(864)	<0·001
0·5	12,593(2373)	<0·01	10,792(1301)	<0·01

inhibited, while that of the dermal fragment was unaffected. When late anagen skin was cultured (day 8; short-term assay: 24 h, 4-mm punches) no significant differences in either Tdr or Arg incorporation were evident between test and control groups. Administering heparin to telogen skin organ culture (day 0; short-term assay, 24 h, 4-mm punches) revealed an additional pattern of response: both epidermal and dermal Tdr incorporation were stimulated by heparin, while both epidermal and dermal Arg incorporation were essentially unaffected by heparin administration to telogen skin *in vitro.*

Heparin administration to mouse epithelial (PAM cells) and to follicle-associated mesenchymal cells (mouse dermal papilla cells) *in vitro* resulted in a significant and dose-dependent inhibition of [^{3}H]-Tdr incorporation as compared to controls (Table 4).

Discussion

Utilizing the C57 Bl-6 model for hair growth studies,[27-30] we report in this pilot study that the systemic administration of heparin inhibits the development of anagen follicles in anagen-induced mice, stimulates epidermal proliferation and arginine-rich protein (ARP) synthesis, and inhibits epithelial bulb cell proliferation. Hair cycle-dependent and differential effects on the latter two parameters were observed when heparin was directly administered to mouse skin grown in organ culture.

Because almost all [^{3}H]-Tdr and [^{3}H]-Arg incorporation into the epidermal and dermal fragment in skin organ culture occurs into epidermal or epithelial bulb keratinocytes, respectively, reflecting the proliferation and differentiation-related protein synthesis by these two keratinocyte populations,[28-30] we conclude the following from our data (Fig. 1, Tables 1–4).

The inhibition of anagen development by heparin in mice is a useful animal model for studying how heparin and other GAG may alter clinically relevant follicular functions and skin physiology in humans (such as heparin-induced alopecia; alopecia mucinosa, mucopolysaccharidoses; anti-angiogenic and anti-tumour properties of heparin).

The inhibition of induced anagen development in mice by heparin administration as reported here is in accordance with earlier observations in rats.[10] Daily subcutaneous injections of very high concentrations of heparin (5–20 mg/kg bodyweight) retarded the spread of the spontaneous wave of hair growth; also, plucking-induced hair growth was slightly inhibited. However, the precision of inducing and the ease of observing any given stage of the hair cycle in C57 Bl-6 mice, the availability of a defined skin organ culture system for *ex-vivo* or *in-vitro* analyses, and the homogeneity of follicle growth wave patterns in mice suggest the use of this model for future studies rather than that of the rat.

The inhibition by heparin of the proliferation of a mouse keratinocyte cell line (PAM cells) and of mouse dermal papilla cells *in vitro* in a dose-dependent fashion suggests that a direct effect of heparin on follicle-associated cells might contribute to the inhibition of anagen follicle development observed *in vivo.* This antimitotic effect of heparin on epithelial and mesenchymal cells *in vitro* agrees with previous studies.[22,23,26]

The epithelial cell response to heparin *in vitro* was dramatically dependent on the stage of the hair cycle (Table 3). We have found that the effects of melanotropin administration on epithelial cell proliferation, using this mouse-skin organ culture assay, were similarly hair cycle-dependent and differed between epidermal and epithelial bulb keratinocytes (unpublished data). The reported fluctuations in rat skin heparin content with a heparin peak in anagen[15,19] suggest that the regulation of hair follicle activity may involve tightly controlled changes in GAG composition, production and receptor expression, characteristic for each stage of the hair cycle.[17,18]

In view of the morphogenic and growth-modulatory properties of GAG[4,7] and the cytokine-modulatory effects of heparin,[39,40] the controlled local release of endogenous heparin by dermal mast cells during early anagen—a period of rapid morphogenesis of a complex mini-organ—may be an important co-factor in the regulation of anagen follicle development. The systemic administration of large doses of exogenous heparin, usually as mixtures of multiple active ingredients, derived from tissues other than the skin and from species other than man or mouse may disturb this process, exert direct antimitotic effects on follicle cells, and alter the synthesis of specific proteins such as ARP, thus leading to hair growth inhibition (rodents) or alopecia (man).

This study characterizes for the first time the physiological, hair cycle-associated patterns of ARP synthesis by epidermal and follicular keratinocytes (Fig. 1). While the incorporation of Arg into the dermal skin fragment probably represents the synthesis of trichohyalin[29,36,37] and is only mildly affected by heparin (Tables 1–3), the nature of the epidermal ARP, whose synthesis appears to be very sensitive to heparin exposure (Tables 1–3), remains to be determined. It is of interest that the peaks of ARP synthesis in the epidermal and dermal fragment were quite distinct and that the dermal peak of ARP synthesis occurred in late anagen just before the onset of catagen, at a time when the proliferative activity of epidermal and epithelial bulb keratinocytes has fallen to very low levels, approaching those of resting skin.[30] The role of ARP in the extensive hair cycle-associated tissue remodelling in mouse skin is being studied, to characterize those ARP biochemically, to determine which ARP species are being produced by the different skin cell populations at what time of the hair cycle, and whether ARP have mainly structural or regulatory functions.

The novel observation that heparin greatly stimulates the synthesis of ARP in the epidermis of early, but not late anagen skin while general protein synthesis may be little affected (Table 3, Experiment A) raises the question of the functional role of epidermal ARP in skin physiology and hair-growth regulation. Heparin may serve as a pharmacological tool for further characterizing these proteins and for elucidating their function. In this context, the induction of epidermal ARP synthesis by heparin may be dependent on the presence of an epidermal wounding response to plucking, which is evident in early but not late anagen skin.[30,34,35]

The finding that follicular ARP synthesis is either unaffected (*ex vivo*, short-term skin organ culture of all cycle stages) or only slightly stimulated by heparin administration (long-term organ culture of early anagen skin) emphasizes another difference between epidermal and follicular keratinocytes: their functional response to exposure to GAG. This supports the notion that epidermal and epithelial bulb keratinocytes represent cell populations with quite different functional properties and biological characteristics.

The contrasting effects of heparin administration on epithelial cell proliferation and differentiation in short-term versus long-term early anagen skin organ culture as well as in telogen and early anagen versus late anagen short-term skin organ culture, require more extensive study before definite conclusions can be drawn (including an extended dose–response curve and short- and long-term incubation schedules in all hair-cycle stages studied). They could indicate, however, that heparin-induced changes in epithelial cell gene expression depend on the length of tissue exposure as well as on cycle-associated fluctuations in epithelial cell responsiveness to this extremely versatile GAG or to other signal molecules modulated by heparin. That heparin/heparan sulphate can for example modulate the presentation of cytokines to other cells as well as their release from storage sites[39,40] might explain why the biological effects of heparin in an organ culture system change over time.

It is quite unlikely that the observed inhibitory effects of heparin can simply be explained by substance toxicity, because the same concentration of heparin had stimulatory effects on keratinocyte functions *in vitro* when choosing different assay protocols using the same model, depending on the stage of the hair cycle, the localization of the respective keratinocyte population and the length of incubation. Also, it was generally the medium range concentration of 0·5 μg heparin/ml that exerted maximal inhibition.

Because heparin is an endogenously occurring GAG, the use of heparin in the combination of *in vivo* and organ culture assays of mature adult follicle populations in their natural tissue environment can be expected to improve our understanding of the role of controlled extracellular matrix alterations in tissue growth regulation. However, because commercially available heparin is a complex and heterogeneous mixture of different molecules, it will be important to use better-defined heparin preparations or synthetic heparinoids in future studies.

One inherent limitation of studying traumatically induced anagen is that it may not in all respects be the same as spontaneous anagen, since plucking induces a wounding response.[30,34,35] At least the mature anagen follicle of plucking induced mouse skin does not show any apparent morphological or functional differences to spontaneously developing anagen follicles.[27,38] Thus, the contrasting results of the effects of heparin as in early anagen versus late anagen skin organ culture may partially reflect a modulation of the wounding response by heparin. Also, it is not clear whether the hair growth-modulatory effects by heparin in our assay system are due to direct effects of this GAG on the hair follicle or whether they may be brought about indirectly via modification of the skin wounding response or other regulatory systems.

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References

1 Oliver RF, Jahoda CAB. Dermal-epidermal interactions. *Clinics Dermatol* 1988; 6: 74–82.
2 *Molecular Biology of the Cell.* (Alberts B *et al.* eds), 2nd edn. New York: Garland, 1989.
3 Uitto J, Olsen DR, Fazio MJ. Extracellular matrix of the skin—50 years of progress. *J Invest Dermatol* 1989; 92 (Suppl): 61S–77S.
4 Ruoslahti E. Proteoglycans in cell regulation. *J Biol Chem* 1989; 264: 13369–72.
5 Link RE, Paus R, Stenn KS *et al.* Epithelial growth by rat vibrissae follicles *in vitro* requires mesenchymal contact via native extracellular matrix. *J Invest Dermatol* 1990; 95: 202–7.
6 Höök M, Kjellen L, Johansson S. Cell-surface glycosaminoglycans. *Ann Rev Biochem* 1984; 53: 847–69.
7 San Antonio JD, Winston BM, Tuan RS. Regulation of chondrogenesis by heparan sulfate and structurally related glycosaminoglycans. *Dev Biol* 1987; 123: 17–24.
8 Rook A, Dawber R. *Diseases of the Hair and Scalp.* Oxford: Blackwell Scientific Publications, 1982.
9 Miki Y. Alopecia from heparin. *Medical J Osaka Univ* 1960; 11: 315–21.
10 Miki Y. The effect of heparin on hair growth in rats. *Med J Osaka Univ* 1960; 11: 325–43.
11 Reed RJ, Clark WH, Mihm MC. The cutaneous mucinoses. *Hum Pathol* 1973; 4: 201–12.
12 Pinkus H. Alopecia mucinosa. *Arch Dermatol* 1957; 76: 419.
13 Langner A, Jablonska S, Darzynkiewicz Z. Studies on the origin of the mucin in mucinosis follicularis. *Acta Derm Venereol (Stockh)* 1969; 49: 76.
14 Braun-Falco O, Theisen H. Über Veränderungen im Hautbindegewebe während des Haarwachstumszyklus der Maus. *Arch klin exp Dermatol* 1959; 209: 426–34.
15 Moretti GM, Ranobou EM, Rebora A. The hair cycle re-evaluated. *Int J Dermatol* 1976; 15: 277.
16 Maekawa Y. Dermal glycosaminoglycan concentration throughout hair growth cycle of rats. *J Dermatol (Tokyo)* 1979; 6: 191–8.
17 Couchman JR, Gibson WT. Expression of basement membrane components through morphological changes in the hair growth cycle. *Dev Biol* 1985; 108: 290–8.
18 Couchman JR, King JL, McCarthy KJ. Distribution of basement membrane proteoglycans through hair follicle development and the hair growth cycle in the rat. *J Invest Dermatol* 1990; 94: 65–70.
19 Moretti G, Giacometti C, Boido V, Rebora A. Histamine, serotonine and mast cells in the skin of the rat during the hair cycle. *J Invest Dermatol* 1963; 40: 205–12.
20 Meyer K, Steigleder K. Effect of acid mucopolysaccharides on hair growth in the rabbit. *Proc Soc Exp Med Biol* 1961; 108: 59–63.
21 Morrison LM, Murata K, Quilligan JJ *et al.* Growth stimulating effects of acid mucopolysaccharides. *Proc Soc Exp Med Biol* 1965; 118: 770–2.
22 Wright TC, Karnovsky MJ. Metabolic effects of heparin on rat cervical epithelial cells. *J Cell Physiol* 1987; 132: 255–62.
23 Scott-Burden T, Buhler FR. Regulation of smooth muscle proliferative phenotype by heparinoid-matrix interactions. *TIPS* 1988; 9: 94–8.
24 Jacques LB. Heparin—a unique misunderstood drug *TIPS* 1982; 3: 289–91.
25 Folkman J. How is blood vessel growth regulated in normal and neoplastic tissue? *Cancer Res* 1986; 46: 467–73.
26 Eskinazi DP, Perna JJ, Ershow AG, Sharrow SO. Effect of heparin on *in vitro* immune parameters. *J Biol Response Mod* 1988; 7: 173–84.
27 Paus R, Stenn KS, Link RE. The induction of anagen hair growth in telogen mouse skin by cyclosporin A. *Lab Invest* 1989; 60: 365–9.
28 Paus R, Stenn KS, Link RE. Telogen skin contains an inhibitor of hair growth. *Br J Dermatol* 1990; 122: 777–84.
29 Paus R, Stenn KS, Elgjo K. Epidermal pentapeptide inhibits hair growth *in vivo* and *in vitro*. *J Invest Dermatol* 1989; 92: 497A.
30 Stenn KS, Paus R, Dutton T. The signal for inducing hair growth is long-lived: the effect of glucocorticoids on hair growth induction in the mouse. *J Invest Dermatol* 1990; 94: 580A
31 Messenger AG. The culture of dermal papilla cells from human hair follicles. *Br J Dermatol* 1984; 110: 685–89.
32 Chase HB, Montagna W, Malone JD. Changes in the skin in relation to the hair regrowth cycle. *Anat Rec* 1953; 116: 75–82.
33 Hansen LS, Coggle JE, Wells J, Charles MW. The influence of the hair cycle on the thickness of mouse skin. *Anat Rec* 1984; 210: 569–73.
34 Argyris TS. Hair growth induced by damage. *Adv Biol Skin* 1967; 9: 339–45.
35 Silver AF, Chase HB, Arsenault CT. Early anagen initiated by plucking compared with early spontaneous anagen. *Adv Biol Skin* 1967; 9: 265–86.
36 Rogers GE. The localization and significance of arginine and citrulline in proteins of the hair follicle. *J Histochem* 1963; 11: 700.
37 Rothnagel JA, Rogers GE. Trichohyalin, an intermediate filament-associated protein of the hair follicle. *J Cell Biol* 1987; 1023: 1419.
38 Slominski A, Paus R, Constantino R. Differential transcription, translation and activity of tyrosinase during induced hair growth in mice. *J Invest Dermatol* 1990, 94: 579A.
39 Burgess AW. Cell surface heparan sulphate implicated in haemopoietic growth factor signalling. *Immunol Today* 1988; 9: 267–8.
40 Folkman J, Klagsbrun M, Sasse J *et al.* A heparin-binding angiogenic protein—basic fibroblast growth factor—is stored within basement membrane. *Am J Pathol* 1988; 130: 393–400.

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Clinical correlations and prognosis based on hyaluronic acid serum levels in patients with progressive systemic sclerosis

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Summary

The serum levels of hyaluronic acid (sHA) were measured using an affinoimmunoenzymatic assay in patients with distal ($n=16$) and proximal ($n=15$) progressive systemic sclerosis (PSS) and in 31 controls. The severity of PSS was evaluated using a standardized organ-involvement score. The mean sHA was significantly higher in the patients with PSS than in controls (mean ± SD: $80 \pm 43{\cdot}4$ μg/l vs. $42{\cdot}3 \pm 19{\cdot}1$ μg/l, $P<0{\cdot}001$). sHA was significantly higher in patients with proximal PSS than in patients with distal PSS ($106{\cdot}4 \pm 44{\cdot}6$ μg/l vs. $55{\cdot}4 \pm 23{\cdot}8$ μg/l, $P<0{\cdot}001$). A positive correlation was found between sHA and the disease score ($r=0{\cdot}67$, $P<0{\cdot}001$). sHA was also correlated with lung diffusion capacity for carbon monoxide ($r=0{\cdot}70$, $P<0{\cdot}001$), but only in those patients who had abnormal lung function, and therefore presumably had lung PSS involvement.

We suggest that sHA could be an indicator of the degree of systemic involvement in PSS. Its prognostic value and possible use in the follow up of patients with PSS remain to be clarified.

Hyaluronic acid or hyaluronan (HA)[1] is a major component of connective tissue, particularly in cartilage and synovial membrane.[2] Recently, it has been reported that glycosaminoglycans (GAG) metabolism was disturbed, and that serum HA (sHA) and urinary GAG excretion were increased in patients with progressive systemic sclerosis (PSS).[3–5] Skin biopsies contained excess GAG, especially in the early oedematous phase of disease;[6] and increased synthesis of hyaluronate was found in cultured skin fibroblasts from patients with PSS.[7]

The aims of our study were to confirm, by using a different method of measurement, that sHA is increased in PSS and to examine the relationship of sHA with the severity of the disease and where there is visceral involvement.

Methods

The serum concentrations of HA (sHA) in patients with PSS and in controls were compared. Thirty-one patients (26 women, five men; mean age $51 \pm 10{\cdot}6$ years, range 31–69 years) with PSS were investigated. Twenty-four (77%) of these patients fulfilled the American Rheumatism Association criteria for systemic sclerosis.[8] Seven patients not fullfilling all those criteria were also included: three had the CREST syndrome (calcinosis, Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly, telangiectasia) and four had an incomplete form of CREST syndrome. In accordance with the criteria of the American Rheumatism Association, patients with PSS were divided into proximal and distal scleroderma subsets.[9] Proximal PSS was defined by extensive skin induration and sclerosis above the metacarpophalangeal joints. All other patients were classified as having distal scleroderma including those not fullfilling the criteria of the American Rheumatism Association.

Proximal PSS (Group 1) consisted of 15 patients (13 women, two men; mean age $50{\cdot}9 \pm 11{\cdot}8$ years; mean duration of disease 12·3 years, range 6–24 years, as determined from the onset of Raynaud's phenomenon; diagnosed since 5·5 years, range 1–8 years). Anti-scleroderma 70 antibody was present in six out of the nine cases. Eight were receiving prednisolone (mean dose 13·5 mg/day; range 8–20 mg), six D-penicillamine (600 mg/day). Twelve patients received a calcium channel antagonist.

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Distal PSS (Group 2) consisted of 16 patients (13 women, three men; mean age 50·1 ± 7 years; mean duration of disease 14·6 years, range 6–27 years; diagnosed since 3·7 years, range 1–8 years). Anticentromere antibody was present in 15 out of 16 patients. In this group, three were on colchicine and all the patients received treatment with a calcium channel antagonist.

All the patients had a standardized evaluation (Table 1) which resulted in a disease score for each patient[10] (Table 2). Oesophageal function was assessed by oesophageal manometry. Lung function was evaluated by chest radiography and determination of total lung capacity, residual volume, vital capacity, forced expiratory volume and lung diffusion capacity for carbon monoxide (DLCO). Renal function was assessed by serum creatinine and 24-h urine collection for protein excretion. Myocardial involvement was assessed from the clinical data and a 12-lead electrocardiograph.

Thirty-one age-matched healthy subjects with no history of rheumatic, liver, renal or thyroid disease served as controls.

All PSS patients and controls satisfied the following criteria: normal liver function tests (alkaline phosphatase < 120 IU/l, alanine amino transferase and aspartate amino transferase < 40 IU/l, gammaglutamyl transpeptidase < 38 IU/l), normal serum creatinine (< 100 μmol/l), absence of proteinuria, absence of antimitochondria autoantibody (by radioimmunoassay) and no clinical evidence for thyroid dysfunction or neoplastic diseases.

The HA measurement was performed as follows. Blood samples were collected between 07:00 and 08:00 h after fasting overnight. HA was assayed by an affinoimmunoenzymatic method as previously described,[11] using hyaluronectin (HN). This glycoprotein, isolated from normal brain tissue, has a strong and specific affinity for HA[12,13] which overcomes the problem of the low immunogenicity of HA. When a solution of hyaluronectin is deposited into the wells of a plate coated with HA, the fixation of hyaluronectin on HA can be demonstrated by an ELISA technique using anti-hyaluronectin antibodies coupled to alkaline phosphatase. When the HN solution is first incubated with a sample the HN attaches itself to sample HA resulting in decreased fixation of HN on the HA adsorbed on the plate. This reduced fixation in turn results in a reduction of light absorption on the spectrophotometer, relative to the quantity of HA present in the sample. Measurement by a calibrated scale of the degree of inhibition of fixation of HN makes it possible to draw a standard inhibition curve showing light absorption in relation to the concentration of HA present in the sample.

Table 1. Disease score

Site of involvement	Criteria	Score
Skin (sclerosis)	Face	1
	Hands	1
	Trunk	1
Gut	Radiological or manometric changes	3
Lungs	Radiological changes and/or abnormal CO transfer factor	3
Heart	ECG changes	3
Kidneys	Proteinuria and/or creatinine clearance < 60 ml/min	3
Other	Sjogren's syndrome, polymyositis	3

Sera were diluted 1:10 in phosphate buffer 0·01 M pH 7·4 with 0·02% sodium azide (PBSA) supplemented with 1 mg/ml streptomyces griseus protease (Sigma, Louis, MO, U.S.A.). They were incubated for 18 h at 37°C then heated at 100°C for 15 min in an oil-bath in order to suppress protease activity. The stock HA solution (1 mg/ml) was treated under the same conditions. Samples and standard solutions were supplemented (v:v) with hyaluronectin 150 μg/l in the same buffer (PBSA), in test tubes. After 1 h, they were incubated in wells of plastic microtest plate (nunc) for 4 h at 4°C. Alkaline phosphatase-conjugated anti-hyaluronectin antibodies were incubated for 18 h at 37°C and the substrate was incubated in wells for 1 h at 37°C. Results were recorded at 405 nm with a Titertek Multiscan (Flow Lab.) and calculations made on a Victor computer. The variability of the assay was tested on 124 determinations of a pooled serum on eight different plates at room temperature. The intra-assay variation coefficient was 6 ± 2·2% and the inter-assay variation coefficient was 12%. On each plate a control serum was incubated to verify the reproducibility of the technique, for which a maximum variation of ± 20% was tolerated.

Statistical tests were carried out with a Macintosh microcomputer, using the SYSTAT™ statistical package. Serum HA levels and disease scores were compared using the Mann–Whitney non-parametric test. Correlations were tested with Spearman's non-parametric test, and quantified with linear regression analysis.

Results

There was no significant difference between the two PSS groups for age, mean duration of disease, or follow up.

The results for sHA are given in Figure 1. Overall, patients with PSS had significantly higher sHA

Table 2. Clinical characteristics of patients

Age (years)	Sex	Visceral involvement	Disease score	Serum HA (μg/l)
Diffuse PSS				
58	M	Skin, oesophagus, lung (LDCO:42%)	8	170
36	F	Skin, lung (LDCO:64%)	5	85
32	F	Skin, lung (LDCO:68%)	5	75
59	F	Skin, oesophagus, lung (LDCO:52%)	9	65
67	F	Skin, oesophagus, lung (LDCO:61%)	9	55
56	F	Skin, oesophagus, lung (LDCO:51%)	8	164
36	F	Skin, oesophagus, lung (LDCO:61%)	9	86
55	F	Skin, lung (LDCO:69%), Gougerot–Sjögren	8	62
61	F	Skin, oesophagus, lung (LDCO:49%)	8	123
41	F	Skin, lung (LDCO:65%), arthralgia	5	82
40	F	Skin, oesophagus, lung (LDCO:56%), Gougerot–Sjögren	11	123
64	F	Skin, oesophagus, lung (LDCO:43%), heart	14	206
56	M	Skin, oesophagus, lung (LDCO:62%)	6	72
51	F	Skin, lung (LDCO:64%), Gougerot–Sjögren	8	112
67	F	Skin, oesophagus, lung (LDCO:56%)	8	117
Limited PSS				
43	M	Skin, oesophagus	4	56
57	F	Skin, lung (LDCO:71%)	5	65
58	M	Skin, lung (LDCO:63%), Gougerot–Sjögren	7	102
60	M	Skin, oesophagus	4	64
49	F	Skin, lung (LDCO:41%)	4	92
51	F	Skin, oesophagus, lung (LDCO:64%)	8	83
59	F	Skin, oesophagus	5	77
58	F	Skin, oesophagus	5	25
69	F	Skin, lung (LDCO:56%)	5	50
48	F	Skin	2	43
44	F	Skin	2	61
43	F	Skin	1	36
44	F	Skin, oesophagus	4	43
37	F	Skin	1	28
52	F	Skin, oesophagus	5	31
31	F	Skin	2	31

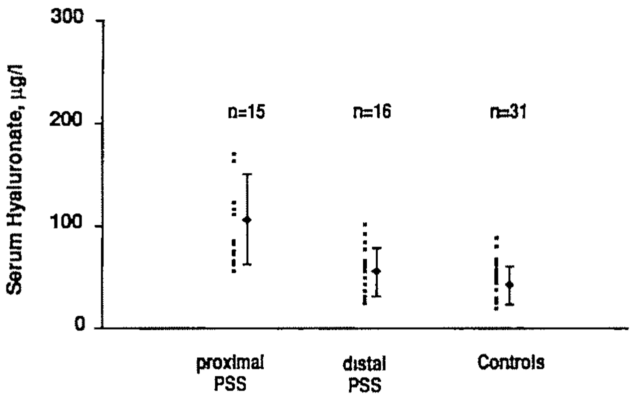

Figure 1. Serum hyaluronate concentrations (μg/l) in proximal and distal progressive systemic sclerosis (PSS) showing individual values (■) and means±SD of patients and 31 controls.

(80·1±43·4 (SD) μg/l, range 25–206) than controls (42·3±19·1 μg/l, $P<0{\cdot}001$). When the patients were subdivided in proximal and distal PSS, only the proximal PSS group had significantly higher mean sHA than controls ($P<0{\cdot}001$). sHA was higher in proximal PSS patients than in patients with distal PSS (106·4±44·6 μg/l, range 55–206, vs. 55·4±23·8 μg/l, range 25–102). The mean sHA of patients with distal PSS was not significantly higher than that of the age-matched healthy controls ($P=0{\cdot}055$).

The median disease score differed significantly ($P<0{\cdot}001$) in the distal and proximal PSS groups (4, range 1–8 and 8, range 5–14, respectively). The HA levels showed a positive correlation with the score of visceral involvement ($r=0{\cdot}67$, $P<0{\cdot}001$), those

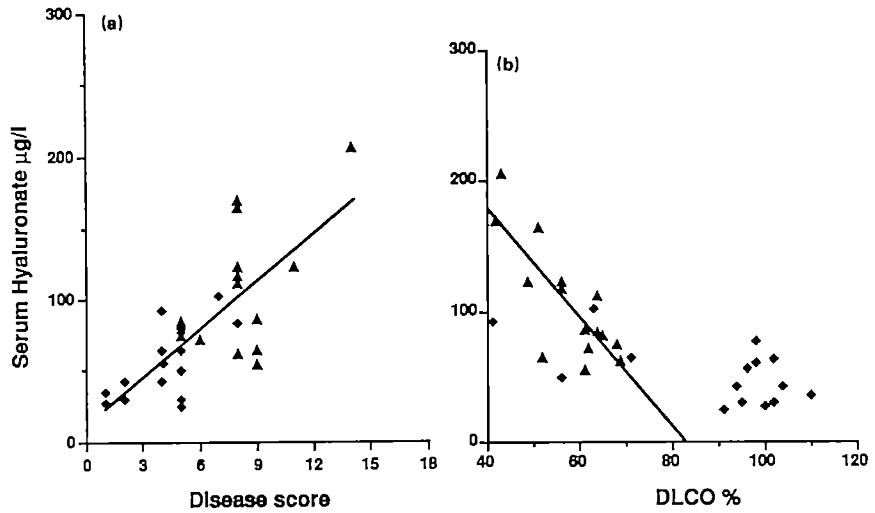

Figure 2. (a) Serum hyaluronate concentrations (µg/l) in proximal and distal progressive systemic sclerosis (PSS): relation with disease score (see Table 1). Overall correlation: $r=0\ 67$ $P<0{\cdot}001$, by Spearman's non-parametric test. Line shows linear correlation (least squares method: $y=12{\cdot}9+11{\cdot}1x$, $r=0{\cdot}74$, $P<0{\cdot}001$). (b) Serum hyaluronate concentrations (µg/l) in proximal and distal progressive systemic sclerosis (PSS): relation with DLCO (% of theoretical value), as an estimator for lung involvement: no correlation in patients with DLCO>80%; for patients with DLCO<80%, $r=0{\cdot}70$, $P<0{\cdot}001$ (Spearman's test); line denotes linear correlation in patients with DLCO<80% (least squares method: $y=346{\cdot}7-4{\cdot}17\ x$, $r=0{\cdot}80$, $P<0{\cdot}001$). ▲, proximal PSS; ◆, distal PSS.

patients with proximal PSS tending to have the highest HA levels (Fig. 2a). When individual types of visceral involvement were studied a correlation was found only with lung involvement. sHA were negatively correlated with DLCO ($r=0{\cdot}70$, $P<0{\cdot}001$) when this was below 80% of theoretical values (all patients in Group 1, and four patients in Group 2) (Fig. 2b).

Discussion

Using a sensitive and specific affinoimmunoenzymatic method, we measured the serum hyaluronic acid concentration in patients with proximal and distal progressive systemic sclerosis. Our results indicate a significant increase of sHA in PSS in the proximal but not the distal type. These results are in accordance with previous hypotheses of disturbed glycosaminoglycans metabolism in this disease. Previous workers have shown an excess glycosaminoglycans content in the skin of patients suffering from PSS,[6] an increased urinary excretion of glycosaminoglycans in PSS[4,5] and a considerable increase in synthesis of hyaluronate by cultured skin fibroblasts from patients with PSS compared with normal skin fibroblasts.[7,14] Our results confirm those of Engström-Laurent *et al.*,[3] obtained using a different assay method. They used a radiochemical assay method where cartilage proteins, having a strong specific affinity for HA labelled with iodine-125, were incubated with a solution of HA and HA linked to agarose gel. This radiochemical method and our affinoimmunoenzymatic assay produce similar results with a good correlation, $r=0{\cdot}97$. Our method could detect high molecular weight HA as well as smaller fragments of HA, down to the HA-derived disaccharide HA 10.[11] Experiments using HA degraded by ferrous ions could not detect any significant difference between native and decayed HA. Thus the expression of HA concentration does not preclude a partial degradation in blood.

The increased levels of serum HA in PSS could be the result of excessive production or decreased catabolism. HA is catabolized only in the endothelial cells of hepatic sinusoids.[15,16] As all our patients had normal liver function and there were no antimitochondrial antibodies, it is probable that as with rheumatoid arthritis[17] and psoriasis,[18] the increase in HA is the result of excessive production.

Increased sHA in PSS patients probably originates from certain affected organs, as is the case for lungs in sarcoidosis[19] or the joints in rheumatoid arthritis.[20] One possible source for increased sHA is the sclerodermatous skin, because raised levels of serum hyaluronate have also been observed in patients with morphoea without involvement of other organs.[3] The excess glycosaminoglycans content in the skin of patients with PSS,[21] the considerable *in-vitro* increase of HA production by cultured skin fibroblasts from patients with scleroderma,[7,14] particularly in response to mitogen-activated T lymphocytes,[22,23] platelet factor,[24] interleukin 1[25] or growth factors[26] all support this hypothesis. Hyaluronate appears to enter the circulation via the lymphatic system[27] and is rapidly eliminated by the endothelial cells of hepatic sinusoids.[15,16]

The raised sHA in patients with PSS could also originate from affected organs other than the skin. Experimental studies have shown that hyaluronate could originate from the joints, and especially from the synovial fluid, which is rich in this glycosaminoglycan.[20,28] These studies have been confirmed by the increase in HA levels in sera from patients suffering from rheumatoid arthritis.[17,29] Excess HA production from joints could be an explanation for raised sHA in some of our patients, but few suffered from joint complaints and only two patients with proximal systemic sclerosis had apparent joint involvement.

The lungs could also be a source because hyaluronate is present in lung parenchyma. Recently Hallgren *et al.*[19] found high concentrations of HA in broncho-alveolar fluid from patients with sarcoidosis who had normal serum values. Sclerodermal lung parenchyma, particularly when there is interstitial fibrosis, could produce hyaluronate as suggested by the correlation we found between sHA and the decrease in DLCO which we used as an indicator of lung involvement. However, the degree of lung involvement could simply indicate more extensive disease, with increased HA production from other sources.

We found a significant increase in HA levels in proximal PSS and a positive correlation between sHA and the disease severity score. This suggests that sHA could be a non-specific indicator of disease severity. Engström-Laurent *et al.*[3] found no relationship between HA levels and serological indicators of inflammatory activity, the extent of the skin lesions or the severity of internal organ involvement. However, they looked for a correlation between HA levels and the degree of involvement of each organ taken alone. PSS is a multi-organ disease, and the condition of a single organ does not necessarily reflect the severity of the overall disease, which should be estimated with a global score of visceral involvement.

We studied a cross-section of PSS forms and related diseases. We believe monitoring of sHA could be used as an indicator of the overall severity of the disease, and, with the clinical disease scores, to evaluate the efficacy of therapy. Although there is are no data on the effects of drugs on sHA in PSS, high-dose steroids reduce increased sHA levels in patients with active rheumatoid arthritis.[29] *In vitro*, cortisol,[30] D-penicillamine[31] and retinoic acid[32] have been shown to inhibit hyaluronic acid production in cultured fibroblasts. Further studies of the usefulness of sHA determination in the management of PSS and other collagen diseases are needed, as are long-term studies of the evolution of sHA in PSS.

In conclusion, we have confirmed the increase of HA levels in PSS, especially in its proximal form. We suggest that hyaluronate levels could be a reflection of the degree of visceral involvement in progressive systemic sclerosis and could be an indicator of the overall severity of the disease. Further studies are required to confirm these findings, to assess the prognostic value of increased sHA in PSS, to investigate the variation in levels of HA as a function of the progress of the disease and to evaluate the effects of treatments on sHA levels.

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References

1 Balazs EA, Laurent TC, Jeanloz RW Nomenclature of hyaluronic acid. *Biochem J* 1986; 235: 903.

2 Meyer K. Biochemistry and biology of mucopolysaccharides. *J Am Med* 1969; 47: 664–72.

3 Engström-Laurent A, Feltelius N, Hallgren R, Wasteson A. Raised serum hyaluronate levels in scleroderma: an effect of growth factor induced activation of tissue cells? *Ann Rheum Dis* 1985; 44: 614–20.

4 Murata K, Takeda M. Compositional changes in urinary acidic glycosaminoglycans in progressive systemic sclerosis. *Clin Chim Acta* 1980; 108: 49–59.

5 Chuck AJ, Murphy J, Weiss JB, Jayson MIV. Urinary glycosaminoglycan excretion in systemic subsets compared with normal controls. *Br J Rheumatol* 1987; 26: 259–61.

6 Fleischmajer R, Perlish J. Glycosaminoglycans in scleroderma and scleredema. *J Invest Dermatol* 1972; 58: 129–32.

7 Bashey RI, Millan A, Jimenez SA. Increased biosynthesis of glycosaminoglycan by scleroderma fibroblasts in culture. *Arthritis Rheum* 1984; 27: 1040–45.

8 Subcommittee for scleroderma criteria of the American Rheumatism Association diagnostic and therapeutic criteria committee: Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum* 1980; 23: 581–90.

9 Steen VD, Powell DL, Medsger TA. Clinical correlation and prognosis based on serum autoantibodies in patients with systemic sclerosis. *Arthritis Rheum* 1988; 31: 196–203.

10 Hughes P, Gelsthorpe K, Doughty RW *et al* The association of HLA-B8 with visceral disease in systemic sclerosis. *Clin Exp Immunol* 1978; 31: 351–6.

11 Delpech B, Bertrand P, Maingonnat C Immunoenzymo-assay of the hyaluronic acid-hyaluronectin interaction: application to the detection of hyaluronic acid in serum of normal subjects and cancer patients. *Anal Biochem* 1985; 149: 555–65.

12 Delpech B, Halavent C. Characterisation and purification from human brain of a hyaluronic acid-binding glycoprotein, hyaluronectin. *J Neurochem* 1981; 36: 855–9.

13 Delpech B. Immuno-chemical characterization of the hyaluronic acid-hyaluronectin interaction. *J Neurochem* 1982; 38: 978–84.

14 Cabral A, Castor CW. Connective tissue activation. XXVII The behavior of skin fibroblasts from patients with scleroderma. *Arthritis Rheum* 1983; 26: 1362–9.

15 Fraser JRE, Laurent TC, Pertoft H. Plasma clearance, tissue distribution and metabolism of hyaluronic acid injected intravenously in rabbit. *Biochem J* 1981; 200: 415–24.

16 Fraser JRE, Laurent TC, Engström-Laurent A. Elimination of

hyaluronic acid from the blood stream in the human. *Clin Exp Pharmacol Physiol* 1984; 11: 12–25.

17 Levesque H, Delpech B, Le Loet X, Deshayes P. Serum hyaluronate in rheumatoid arthritis: study by affinoimmunoenzymatic assay. *Br J Rheum* 1988; 27: 445–9.

18 Lundin A, Engström-Laurent A, Hallgren R *et al.* Circulating hyaluronate in psoriasis *Br J Dermatol* 1985; 112: 663–71.

19 Hallgren R, Eklund A, Engstrom-Laurent A, Schmekel B. Hyaluronate in bronchoalveolar lavage fluid· a new marker in sarcoidosis reflecting pulmonary disease. *Br Med J* 1985; 290: 1778–81.

20 Dahl IM, Husby G. Hyaluronic acid production *in vitro* by synovial lining cells from normal and rheumatoid joints. *Ann Rheum Dis* 1985; 44: 647–57.

21 Uitto J, Ohlenschlager K, Lorenzen IB. Solubility of skin collagen in normal human subjects and in patients with scleroderma *Clin Chim Acta* 1971, 31: 13–18.

22 Whiteside TL, Worral JG, Prince RK *et al* Soluble mediators from mononuclear cells increase the synthesis of glycosaminoglycan by dermal fibroblast cultures derived from normal subjects and progressive systemic patients. *Arthritis Rheum* 1985; 28: 188–97.

23 Bocquet J, Langris M, Daireaux M *et al.* Mononuclear cell-mediated modulation of synovial cell metabolism: increased hyaluronic acid synthesis by a monocyte cell factor (MCF) *Exp Cell Research* 1985; 160: 9–18.

24 Falanga V, Alstadt SP. Effect of a platelet release fraction on glycosaminoglycan synthesis by cultured dermal fibroblasts from patients with systemic sclerosis. *Br J Dermatol* 1988, 118: 339–45.

25 Falanga V, Tiegs SL, Alstadt SP *et al* Transforming growth factor-beta. selective increase in glycosaminoglycan synthesis by cultures of fibroblasts from patients with progressive systemic sclerosis. *J Invest Dermatol* 1987; 89: 100–4.

26 Postlethwaite AE, Smith GN, Lachman LB *et al.* Stimulation of glycosaminoglycan synthesis in cultured human dermal fibroblasts by interleukin 1. *J Clin Invest* 1989; 83: 629–36.

27 Laurent UBG, Laurent TC. On the origin of hyaluronate in blood *Biochem Int* 1981; 2: 195–9.

28 Vuorio E, Einola S, Hakkarainen S. Synthesis of underpolymerized hyaluronic acid by fibroblasts cultured from rheumatoid and non rheumatoid synovitis. *Rheumatol Int* 1982; 2: 97–102.

29 Engström-Laurent A, Hallgren R. Circulating hyaluronate in rheumatoid arthritis: relationship to inflammatory activity and the effect of corticosteroid therapy. *Ann Rheum Dis* 1985; 44: 83–8.

30 Moscatelli D, Rubin H. Hormonal control of hyaluronic acid production in fibroblasts and its relation to nucleic acid and protein synthesis. *J Cell Physiol* 1976; 91: 79–88.

31 Priestley GC. Changes in the growth and metabolism of cells cultured from normal, sclerotic and rheumatoid connective tissue brought about by D-penicillamine and by sodium salicylate. *J Invest Dermatol* 1980; 74: 413–7.

32 Smith TJ. Retinoic acid inhibition of hyaluronate synthesis in cultured human skin fibroblasts. *J Clin Endocrinol* 1990; 70: 655–60.

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The effect of treatment with 13-*cis*-retinoic acid on the metabolic burst of peripheral blood neutrophils from patients with acne

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Summary The N-formylmethionyl-leucyl-phenylalanine (f-MLP)-induced metabolic burst activity of peripheral blood neutrophils isolated from acne patients undergoing treatment with 13-*cis*-retinoic acid at a dose of 1·0 mg/kg/day was investigated using a luminol-enhanced chemiluminescence assay. The mean and median chemiluminescence response were significantly greater ($P<0{\cdot}05$) in patients receiving 13-*cis*-retinoic acid than in untreated acne patients or age-matched controls. Pre-incubation of neutrophils with 13-*cis*-retinoic acid (10 nmol/l) did not affect the chemiluminescence response to formyl peptide. A sequential study over 20 weeks in seven patients demonstrated that chemiluminescence peaked after 2–8 weeks of treatment. In three patients this was accompanied by a worsening of their acne. These studies suggest that, in the initial phase, treatment with 13-*cis*-retinoic acid may exacerbate, through pro-inflammatory priming of neutrophils, certain neutrophil-mediated inflammatory processes in acne.

13-*cis*-retinoic acid is recognized as an effective therapy for nodulocystic and severe acne with predictable and generally reversible side-effects. In doses ranging from 0·1 to 2·0 mg/kg body weight it causes a marked reduction in sebum excretion rate, which persists for some time after therapy has been completed.[1] In a similar range of doses it has effects on follicular keratinization,[2] on the microflora of the pilosebaceous unit and on the skin as a whole.[3]

Modulation of inflammatory processes has also been thought to be a factor in the therapeutic action of 13-*cis*-retinoic acid and this may be via actions on neutrophils. The chemotactic response of neutrophils has been studied both *in vivo* and *in vitro*. *In vivo* the pustular response to potassium iodide patch tests[4] and migration into microchambers containing autologous zymosan-activated serum[5] has been shown to be reduced during therapy with 13-*cis*-retinoic acid. Chemotaxis was not shown to be inhibited *in vitro* except at suprapharmacological doses.[6] However similar findings were also reported for superoxide anion and neutrophil enzyme release.[7]

Tissue damage by neutrophils in inflammation is mediated by membrane-derived superoxides and granule-derived enzymes such as myeloperoxidase, working both independently and in co-operation. Superoxide ion generation and myeloperoxidase release is termed the 'metabolic burst' and is precipitated by a number of pro-inflammatory stimuli both *in vivo* and *in vitro*. The synthetic peptide N-formylmethionyl-leucyl-phenylalanine (f-MLP) is well recognized, *in vitro*, as a potent pro-inflammatory stimulus.[8,9] The metabolic burst activity can be quantified by measurement of the chemiluminescence generated by the interaction of the reactive oxygen species with luminol.[8]

In this study we have investigated the possibility that 13-*cis*-retinoic acid may modulate the responsiveness of peripheral blood neutrophils to pro-inflammatory stimuli. Neutrophils isolated from acne patients receiving 1 mg/kg/day 13-*cis*-retinoic acid, acne and non-acne controls were stimulated by f-MLP and the metabolic burst measured by a luminol-enhanced chemiluminescence assay.[8]

Methods

Subjects

Local ethical committee approval was obtained for this study. The subjects were divided into three groups as follows. Group 1, treatment group: 28 patients (17 male,

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11 female, aged 16–41 years) with severe or nodulocystic acne resistant to other therapies were sampled on a total of 39 occasions during treatment with 13-*cis*-retinoic acid 1·0 mg/kg/day. Sampling times varied from 2 weeks to 4 months during treatment.

Group 2, untreated controls: 21 patients with acne of a severity thought to warrant therapy with 13-*cis*-retinoic acid. Seven of these patients later went on to therapy and were included in Group 1.

Group 3, non-acne controls: 24 volunteers age- and sex-matched to Group 1.

No subjects were receiving other medication prior to entering the study and seven patients were followed longitudinally prior to and during therapy.

Neutrophil chemiluminescence assay

Isolation of neutrophils. Neutrophils were prepared from 20 ml of freshly drawn heparinized venous blood by dextran/Ficoll-Hypaque separation.[9] Briefly, specimens with 10 U/ml lithium heparin were allowed to stand with dextran 4 ml for 40–50 min at 37°C. The supernatant was then layered over 10 ml Ficoll-Hypaque (Pharmacia Fine Chemicals, Sweden) and centrifuged for 30 min at 700 *g*. The cell pellet was washed in cation-free saline, centrifuged for 5 min at 700 *g* and erythrocytes in the pellet lysed with 0·5 ml distilled water for 30 s. After a further wash, cells were resuspended in saline with divalent cations added, filtered through 10-μm mesh Nitex, counted and the cell concentration adjusted to 2×10^6/ml. For the assay, 0·5 ml of this cell stock was used to give a final cell concentration of 1×10^6/ml.

Luminol-enhanced chemiluminescence assay. The chemiluminescence response of freshly isolated neutrophils to f-MLP was measured in a 25-tube capacity L.K.B. 1251 luminometer, which was controlled by a microcomputer. A peristaltic pump delivered 100 μl of a cocktail containing f-MLP (10^{-6}M), luminol (2×10^{-4}M) and cytochalasin B (2 μg/ml) to the sample tubes containing neutrophils at the start of the assay. The final volume of samples was 1 ml. Three or four replicates were assayed from each neutrophil preparation. Light output (Mv) was measured over 1 h. The results are presented as the means of summed outputs from replicate tubes.

In another experiment 13-*cis*-retinoic acid (10^{-8}M) was added to sample tubes containing neutrophils for a pre-incubation period of 60 min before addition of the chemiluminescence reagents.

Statistical analysis

Mean and median chemiluminescence responses were calculated for each group. Group means were compared by the Student's *t*-test and medians by Mann–Whitney analysis. Differences were considered statistically significant at $P<0·05$.

Results

Pre-incubation of neutrophils *in vitro* with 10^{-8}M 13-*cis*-retinoic acid did not influence the chemiluminescence response to formyl peptide in any of the groups studied. The chemiluminescence response to stimulation by formyl peptide of neutrophils isolated from each of the three patient groups is demonstrated in Figure 1. The mean and median response in the acne patients receiving 1·0 mg/kg/day 13-*cis*-retinoic acid (Group 1) were 119·53 (+13·1 SEM) and 100·05 (range 25·14–362·85), respectively. The mean and median chemiluminescence response in Group 2 (acne patients not receiving 13-*cis*-retinoic acid) were 71·4 (+14·5 SEM) and 45·82 (range 18·2–275·65) respectively. In Group

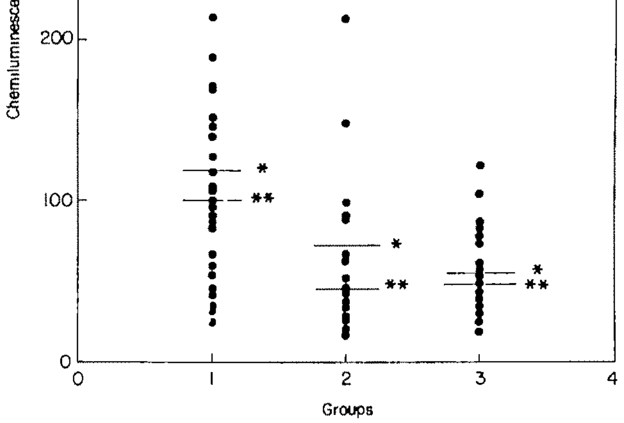

Figure 1. Chemiluminescence response Mv in acne patients receiving 13-*cis*-retinoic acid (Group 1), acne patients not on therapy (Group 2) and non-acne controls (Group 3). Mean (*) and median (**) chemiluminescence responses are shown for each group There were statistically significant differences between Group 1 and both Groups 2 and 3, but no difference between Groups 2 and 3.

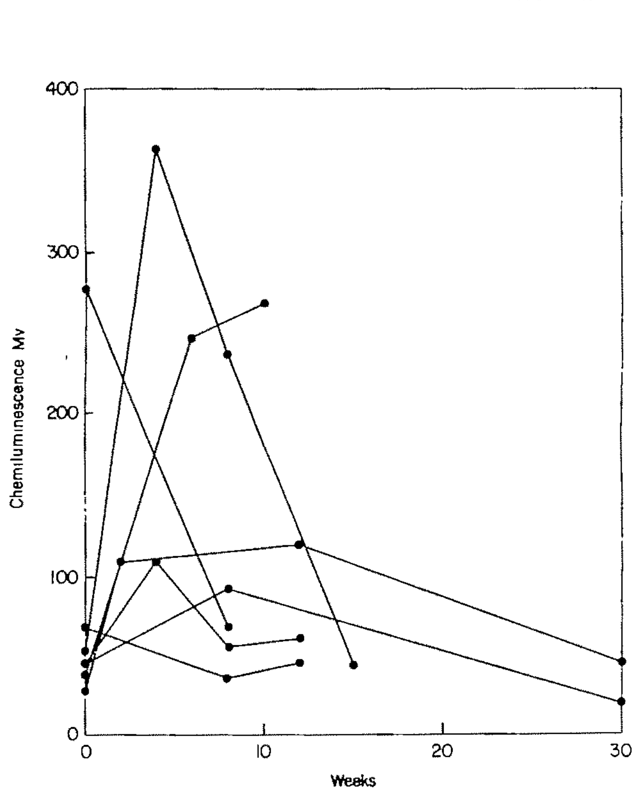

Figure 2. Chemiluminescence response versus time on therapy with 13-*cis*-retinoic acid in the seven patients for whom sequential data was available. Each line indicates an individual patient. All patients finished therapy at or before 20 weeks and the two values at 30 weeks are post-therapy.

3 (non-acne controls) the mean chemiluminescence response was 55·52 (+4·82 SEM) and median 48·45 (range 18·91–121·79).

Significant differences of $P < 0{\cdot}03$ and $P < 0{\cdot}01$ were found when comparing the on-treatment group (Group 1) with the acne control group (Group 2) using the Student's *t*-test and Mann–Whitney comparison, respectively. A significantly greater chemiluminescence response ($P < 0{\cdot}001$) was also seen when the treatment group was compared with the non-acne control group using both parametric and non-parametric analysis as before. No significant differences were noted between the two control groups (Group 2 and Group 3) using either of the statistical tests.

In the seven patients for whom sequential data were available, six patients had a rise in chemiluminescence response following initiation of therapy (Fig. 2). This was followed by a reduction in response in those for whom data was available. The rise occurred after 2–8 weeks of therapy in these cases. Three of these patients developed an exacerbation of their acne in the early stages of treatment as determined by their acne grade,[10] with one patient developing acne in a previously unaffected area.

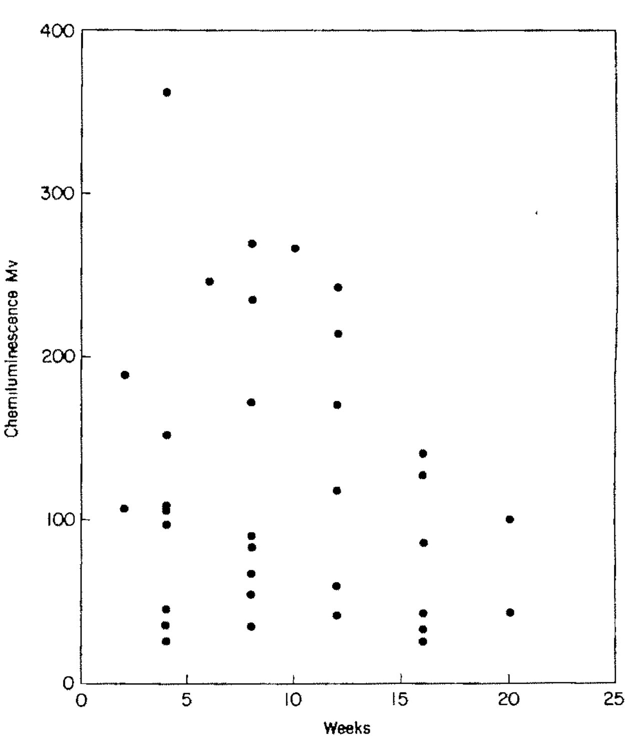

Figure 3. Chemiluminescence response in Group 1 versus time on therapy with 13-*cis*-retinoic acid. $r = 0{\cdot}10$, not significant

All patients eventually had a satisfactory response to treatment irrespective of their chemiluminescence response or initial flare of acne.

Cross-sectional analysis for the whole of Group 1 comparing chemiluminescence response to time on treatment suggested an inverse relationship but this failed to reach statistical significance (Fig 3, $r = 0{\cdot}10$).

Discussion

Prior to taking part in inflammatory processes neutrophils must migrate from the intravascular space to the site of inflammation which in acne is the pilosebaceous unit. Migration is initiated by chemokinetic factors produced both by *Proprionibacterium acnes* and host responses. *In-vivo* evidence of a reduction in neutrophil chemotaxis has been suggested by the reduction in pustule formation following potassium iodide patch tests in patients receiving 13-*cis*-retinoic acid.[4] This was supported by a reduced response to autologous zymosan-activated serum in an *in-vivo* assay of neutrophil chemotaxis.[5] However two other studies[6,7] have failed *in vitro* to demonstrate a reduction in chemotaxis except at suprapharmacological doses (10^{-5}M). These studies have also reported a reduction in superoxide anion production[6,7] after *in-vitro* incubation with high concentrations of 13-*cis*-retinoic acid. In contrast to the above studies which suggest that 13-*cis*-retinoic acid may suppress inflammatory reactions by inhibiting neutrophil invasion of tissues and influencing the metabolic burst, the present study has provided evidence that this drug may enhance neutrophil responses to pro-inflammatory stimuli.

Peripheral blood neutrophils isolated from patients receiving 1 mg/kg/day 13-*cis*-retinoic acid appear to be 'primed'. It may be that 13-*cis*-retinoic acid exerts transcriptional effects on neutrophils relatively early in their development which enhance their response to pro-inflammatory stimuli. However, there are many instances in which priming of neutrophils have been demonstrated. These include exposure to other pro-inflammatory stimuli,[11,12] inflammatory dermatoses characterized by neutrophil invasion,[13] pustular psoriasis[14] and bacterial infections.[15] In some cases[11,12] this occurs in mature neutrophils and developmental effects seem unlikely.

Ideally all the patients would have had their chemiluminescence response measured prior to starting the therapy and during therapy but this was not practicable. In the patients studied sequentially over 20 weeks an initial increase in chemiluminescence response was followed by a reduction. It was related in three of the patients to an increase in the severity of their acne prior to an improvement. Worsening of acne in the early stages of treatment with 13-*cis*-retinoic acid has been reported previously,[16–18] particularly during the early clinical trials.

In the initial stages of therapy with 13-*cis*-retinoic acid there are major changes occurring in the micro-environment of the pilosebaceous unit. It has been suggested that the decrease in the sebaceous gland lipid production may be accompanied by the death of *P. acnes* and the release of inflammatory mediators.[19] These could act to prime neutrophils, which would be consistent with our observations.

References

1 Jones DH, Greenwood R, Cunliffe WJ. The dose response relationship of sebum suppression by 13-*cis*-retinoic acid in severe acne. *Br J Dermatol* 1983; 109: 366.

2 Jones DH, Cunliffe WJ, Holmes PL, Al-Baghdadi H. Effect of 13-*cis*-retinoic acid on pilosebaceous duct obstruction. *Br J Dermatol* 1982; 107(Suppl. 22): 35.

3 Jones DH, Holland KT, Cunliffe WJ. Effect of 13-*cis*-retinoic acid and erythromycin on facial surface and follicular skin microflora in nodulocystic acne. *Br J Dermatol* 1983; 109: 697.

4 Plewig G, Wagner A. Anti-inflammatory effects of 13-*cis*-retinoic acid. An *in vivo* study. *Arch Dermatol Res* 1981, 270: 89–94.

5 Norris DA, Osborn R, Robinson W, Tonnesen MG. Isotretinoin produces significant inhibition of neutrophil chemotaxis *in vivo* in patients with cystic acne. *J Invest Dermatol* 1987; 89: 38–43.

6 Shalita AR, Lee WL. Effects of retinoids on neutrophils. *Clin Res* 1985; 33: 302.

7 Camisa C, Eisensta B, Ragaz A, Weissmann G. The effect of retinoids on neutrophil function *in vitro*. *J Am Acad Dermatol* 1982; 6(Suppl.): 620–9.

8 Lackie JM, Lawrence AJ. Signal response transduction in rabbit neutrophil leucocytes. The effect of exogenous phospholipase A2 suggest two pathways exist. *Biochem Pharmacol* 1987; 36: 1941–5.

9 Boyum A Isolation of mononuclear cells and granulocytes from human blood: Isolation of mononuclear cells by one centrifugation and of granulocytes by combining centrifugation and sedimentation at 1 *g*. *Scand J Clin Lab Invest* 1968, 97(Suppl.): 77–89.

10 Burke BM, Cunliffe WJ. The assessment of acne vulgaris: the Leeds technique. *Br J Dermatol* 1984; 111: 83–92.

11 Bender JG, McPhail LC, Van Epps DE. Exposure of human neutrophils to chemotactic factors potentiates activation of the respiratory burst enzyme. *J Immunol* 1983; 130: 2316.

12 English D, Roloff JS, Lukens JN. Chemotactic factor enhancement of superoxide release from fluoride and phorbol myristate acetate stimulated neutrophils. *Blood* 1981; 58: 129–34

13 De Simone C, De Sole P, Di Mario G *et al.* Reactive oxygen species production in circulating polymorphonuclear leucocytes in psoriasis. *Acta Derm Venereol (Stockh)* 1989; 146(Suppl.): 80–2.

14 Coble BI, Dahlgren C, Molin L, Stendahl O. Neutrophil function in psoriasis: effects of retinoids. *Acta Derm Venereol (Stockh)* 1987; 67: 481–90.

15 Follin P, Briheim G, Sandstedt S, Dahlgren C. Human neutrophil chemiluminescence and f-meth-leu-phe receptor exposure in bacterial infections. *APMIS* 1989; 97: 585–90.

16 Goldstein JA, Comite H, Mescon H, Pochi PE. Isotretinoin in the treatment of acne. Histologic changes sebum production and clinical observations. *Arch Dermatol* 1982; 118: 555–8.

17 Katz RA, Jorgensen H, Nigra TP. Flare of cystic acne from oral isotretinoin. *J Am Acad Dermatol* 1983; 8: 132–3

18 Shalita AR, Cunningham WJ, Leyden JJ *et al.* Isotretinoin treatment of acne and related disorders: An update. *J Am Acad Dermatol* 1983; 9: 629–38.

19 Cunliffe WJ *Acne*. Martin Dunitz, 1989, 311.

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Acute infectious erythemas in children: a clinico-microbiological study

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Summary One-hundred children with an acute illness comprising fever and widespread erythematous rash were prospectively studied to determine whether clinical presentations are helpful in defining the causative agent and to identify the most appropriate microbiological specimens. An infectious agent was identified in 65 children; 72% were viruses, 20% were bacteria, 5% were *Mycoplasma pneumoniae* and in 3% both viruses and bacteria were detected. The most common infectious agents were picornaviruses, an atypical presentation of measles and Group A β-haemolytic *Streptococcus*. Different patterns of rash occurred with each of these infections. The clinical presentation of a child with an acute febrile illness and rash was unhelpful in defining the causative agent. Routine management should include a throat swab for bacterial investigation and in selected cases a blood sample for IgM viral titres.

Children frequently present either to general practitioners or paediatricians with an acute febrile illness and a rash of short duration, presumed to be due to a viral infection, but which does not conform to any specific diagnosis. The usual approach is reassurance and to prescribe paracetamol. The subject of viral rashes was reviewed by Cherry[1] in 1969 and Wenner[2] in 1973. However, since then, laboratory techniques have advanced considerably and it is now possible to identify a wider range of infectious agents. It is important to attempt to diagnose these rashes as more appropriate advice can then be given regarding the nature of the illness, time off school, immunizations and whether the illness is a risk to pregnant women and to other contacts, especially leukaemic and immunocompromized children.

Methods

One-hundred children were seen in the casualty department at Queen Elizabeth Hospital (QEH) with a diagnosis of 'rash associated with a viral infection' from September 1988 to August 1989. They were either general practitioner or self-referrals to casualty. QEH serves a socially deprived inner city catchment area in East London.[3,4] Criteria for entry into the study were an acute erythematous rash and a febrile illness of short duration. Children with recognizable rashes such as classical measles and chicken-pox were excluded from the study.

The following details were noted in each case: age, associated symptoms, drug history, contacts, immunizations and past medical history. A general examination of the child was performed. The rash was documented using body diagrams and standard view photographs whenever possible. Laboratory samples taken in each case included: a throat swab in charcoal transport medium for bacterial investigation (TSB); a throat swab in viral transport medium (TSV); stool or rectal swab in viral transport medium (SV), urine for virology (UV); vesicle fluid (VF) if available and 5 ml of clotted blood (CB). Most children were well enough to go home although some required hospital admission. A 1-month follow-up appointment was given to all children for repeat assessment and if necessary further serum samples.

Laboratory investigations

All specimens were initially refrigerated at 4°C, and processed within 24 h. Serum samples were then stored at -20°C and all other samples at -70°C after inoculation. The throat swab (TSV), rectal swab/stool, vesicle fluid and some of the urine sample were inocu-

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lated into human embryonic lung, Vero's (continuous African green monkey kidney cells) and baboon kidney cell lines. Two tubes of each culture medium were kept for 4 weeks or until a cytopathic effect was observed. They were examined on alternate days. Baboon kidney tissue cultures were routinely tested for haemadsorption with human 'O' cells during the second and third week of inoculation. Further identification of virus types was carried out using neutralization tests as previously described.[5] Bacterial culture of throat swabs was carried out on blood agar, Hoyles and Sabouraud's plates. Smears were made from sedimented urinary cells and were examined for measles virus by indirect immunofluorescence (IF) using the technique of Llanes-Rodas and Liu.[6] The following serological tests were performed: *Mycoplasma pneumoniae* total antibody (Serodia-myco 11 kit from Fujirebio via Mast Diagnostics, Merseyside, U.K.) and IgM (IF at the Mycoplasma Reference Laboratory, Central Public Health Laboratory, Colindale (CPHL, London, U.K.); hepatitis B surface antigen (Wellcozyme Enzyme immunoassay (EIA) from Wellcome Diagnostics, Dartford, Kent, U.K.); measles IgM (Sorin E1A Biomedica, Ely, Cambs, U.K.); rubella IgM (E1A using Northumbria Biologicals Ltd (Cramlington, Northumberland, U.K.) or Division of Microbiological Reagents and Quality Control (DMRQC), CPHL reagents with positive sera confirmed by M antibody capture radioimmunoassay (MACRIA)); monospot (infectious mononucleosis absoption kit, Mercia Diagnostics (Guildford, Surrey, U.K.), with positive sera being further tested for Epstein–Barr virus IgG and IgM by IF); hepatitis A IgM (DMRQC CPHL reagent); ASO titre (Rapidia-ASO from Fujirebio via Mast Diagnostics); parvovirus IgM (Virus Reference Laboratory (VRL), CPHL), enterovirus IgM (E1A Epsom PHL). The above investigations were carried out in all 100 patients. In addition, 12 paired serum samples were tested for human herpes virus type 6, HHV6 (VRL, CPHL).

Results

The study children consisted of 54 males and 46 females; aged 1 month to 13 years 5 months; 85% were less than 5 years and 43% were less than 1 year. In 85 of the children, the rash had been present for less than 2 days, with a range of onset from 2 h to 5 days.

All of the children were miserable and febrile and most had a malar flush. In the majority, the rash presented on the trunk with extension to the face, neck and limbs, depending on the length of illness. Nine different patterns of rash were observed (Table 1). The fine macular, maculopapular and papular rashes were defined as discrete lesions which were less than 2 mm in diameter. Papular acrodermatitis (Gianotti–Crosti syndrome) was characterized by coppery-red papules, mainly on the distal arms and legs, spreading to the buttocks and face. Urticarial eruptions were seen as a transient fleeting erythematous rash, characterized by the presence of raised weals with individual lesions fading within 2–6 h and subsequently reappearing elsewhere on the body. The most common presentations of rash were types II, IV, V and I respectively.

Table 1. Classification of acute infectious erythemas

Type of rash		No. of patients
I	Fine macular erythema	15
II	Large/confluent macular erythema	21
III	Fine papular erythema	9
IV	Fine maculopapular erythema	21
V	Large/confluent maculopapular erythema	18
VI	Urticarial rash	5
VII	Erythema with microvesicles	8
VIII	Erythema with petechiae	1
IX	Papular acrodermatitis (Gianotti–Crosti)	2
Total		100

In 65 children (37 males (57%) and 28 females (43%)) infectious agents were identified (Table 2); 47 were viruses (17 types), 13 were bacteria (*Streptococcus* or *Staphylococcus*), three were *Mycoplasma pneumoniae* and in two related children both viruses and bacteria were found (by detection of *Enterovirus* IgM and Group A *Streptococci* culture). Twenty-eight of the 65 children (43%) with a single identified infectious agent had positive results from two or more laboratory samples. The most common infectious agents were picornaviruses (identified as echovirus, coxsackie and *Enterovirus* IgM) in 14 patients, an atypical clinical presentation of measles in 12 patients and Group A β-haemolytic *Streptococcus* in 11 patients. A variety of different patterns of rash were witnessed in each of these groups. However, it was of interest that there was some specificity for the echovirus and coxsackie infections, with only one pattern of rash for each subtype; although the number of patients in each of these groups was small.

Eight children who presented with widespread patchy erythema subsequently developed localized areas of microvesicles: two of these children had coxsackie A9 identified in the stool; two had Group A β-haemolytic *Streptococcus* cultured from throat swab (TSB) together with high ASO titres; two developed frank blistering 12 h

Table 2. Clinico-microbiological results

Infectious agent	Number of patients	Age range (years)	Clinical presentation of rash*	Positive microbiological specimens†
Measles	12	0·3–9·5	I(1); II(2); III(3); IV(3); V(2); VIII(1)	CB(12) UV(7)
Group A‡ *Streptococcus*	11	2·2–9·8	I(1); II(3); III(1); IV(1); V(2); VII(2); IX(1)	TSB(10) CB(11)
Rubella	7	0 4–5·7	I(1); III(1); IV(2); V(2); IX(1)	CB(7)
Echovirus				
Type 2	1	1·2	IV(1)	TSV; CB
5	2	0 3–0 8	I(2)	SV(2); CB(2)
9	2	0·1–0·5	IV(2)	SV(1); CB(2)
16	1	1·5	II(1)	SV; CB
25	1	0·6	VI(1)	SV; TSV; CB
Mycoplasma pneumoniae	3	0·5–10·8	II(2); VI(1)	CB(3)
Respiratory syncytial virus	3	0·2–1 2	I(2); II(1)	NPA(2); TSV(2)
Epstein–Barr virus	3	1·7–4·7	IV(1); V(2)	CB(3)
Coxsackie				
Type A9	2	2·0–4·3	VII(2)	SV(2)
A16	1	1·2	VI(1)	SV; CB
Varicella	2	0·9–4·3	VII(2)	VF(2)
Hepatitis A	2	6·8–7·1	II(1); V(1)	CB(2)
Adenovirus				
Type 3	1	3·8	III(1)	TSV
7	1	0·8	V(1)	TSV
Parvovirus	1	7·2	II(1)	CB
Rotavirus	1	0·5	V(1)	SV
Staphylococcus aureus	1	1·0	V(1)	TSB; SB
Group G *Streptococcus*	1	0·5	I(1)	TSB
Enterovirus IgM	4	0·2–1·3	II(1), IV(2); VI(1)	CB(4)
Group A *Streptococcus* and enterovirus IgM	2	2·7–4·2	III(2)	TSB(2); CB(2)
Undiagnosed	35	0·1–13·4	I(7); II(9); III(1); IV(9); V(5); VI(2), VII(2)	—

* I, fine macular; II, large/confluent macular; III, fine papular; IV, fine maculopapular; V, large/confluent maculopapular; VI, urticarial; VII, erythema with microvesicles; VIII, erythema with petechiae; IX, papular acrodermatitis (Gianotti–Crosti)

† TSB, throat swab for bacteriology; TSV, throat swab for virology; UV, urine for virology; SV, stool or rectal swab for virology; CB, clotted blood; NPA, nasopharyngeal aspirate; SB, skin swab for bacteriology.

‡ One child was subsequently diagnosed as having Kawasaki's disease (see text).

Figures in parentheses represent the number of patients.

after presentation, clinically compatible with chickenpox which was subsequently confirmed by identification and culture of the varicella virus in vesicle fluid; and two remained undiagnosed.

One child had a generalized erythematous rash with widespread petechiae, which was serologically confirmed as an acute measles infection. His platelet count was normal, although initially he was thought to have idiopathic thrombocytopenic purpura. Two children had hepatitis B negative papular acrodermatitis (Gianotti–Crosti syndrome).

Infections identified in the younger age group (less than 1·5 years) included four children with measles, two with rubella, seven with echovirus, four with enteroviral infections diagnosed by detection of IgM, three with respiratory syncytial virus infections and one child with rotavirus infection. The relevance of rotavirus with regard to the rash is questionable. All the children with Group A β-haemolytic *Streptococcus* were older than 2 years.

History and associated clinical features such as runny nose, cough, arthralgia and diarrhoea were not always indicative of the type of infection. Although four children with enteroviral infections had diarrhoea, this symptom was also present with Group A β-haemolytic *Streptococcus*, measles and rubella. Two of the children with respiratory syncytial virus were wheezy, but one child had no respiratory symptoms. One child with hepatitis A had diarrhoea and was mildly jaundiced; the other child with hepatitis A was anicteric. Two of the three children with Epstein–Barr infection (infectious mononucleosis) had received ampicillin prior to the onset of their rash; the other child had no history of recent antibiotic therapy.

Forty-one cases presented between April and June with the remaining cases being equally distributed throughout the year. A peak of rashes associated with measles was present in winter and with enteroviral infections in the spring and summer months. Group A β-haemolytic *Streptococcal* infections were seen throughout the year.

Fourteen children had bacteria identified from throat swabs with a moderate to heavy growth of Group A β-haemolytic *Streptococcus* in 12 children, Group G *Streptococcus* in one child and *Staphylococcus aureus* in one child. The predominant M types of Group A β-haemolytic *Streptococcus* were 3, 4, and 12. In the child with *S. aureus*, this was also identified from multiple skin swabs: the *Staphylococci* typed strongly as bacteriophage type 95 at 100 RTD and produced enterotoxin C (negative results were obtained when the *Staphylococci* were tested for the production of exfoliative toxins A and B).

Viruses were detected by: blood IgM titres in 44 children; throat swab for viral culture in 12 children and vesicle fluid in two children. Measles was confirmed serologically in 12 patients. Urine was useful for detecting measles virus by immunofluorescence in all seven girls but gave negative results in the five boys with confirmed measles. This is presumably due to the increased number of epithelial cells in the urine of female patients. Screening for HHV6 gave no evidence of current or recent infections in the 12 patients that were tested.

All children with bacterial infections were treated with appropriate antibiotics. Eighty-one children were sent home from casualty. Nineteen children were admitted (age range 0·1–9·7 years, mean age 2·2 years). Of the children admitted to hospital, one child with a macular erythematous rash, included in Group II, was diagnosed as having Kawasaki's disease together with a recent Group A β-haemolytic *Streptococcus* infection (raised ASO titre). He developed peeling of his fingertips and subsequent generalized desquamation of skin. Investigations revealed a platelet count of greater than 1000×10^9/l, markedly elevated transaminases, an enlarged gall-bladder on ultrasound examination and coronary artery dilatation seen on echocardiography. Treatment consisted of intravenous benzylpenicillin, a 5-day course of immunoglobulins, oral aspirin and dipyridamole. The other children who were admitted included a child with Group A β-haemolytic *Streptococcus* and a migratory arthralgia; three children with bronchiolitis associated with respiratory syncytial virus who needed tube feeding and oxygen therapy; one child with cystic fibrosis and anicteric hepatitis A and the two children with Gianotti–Crosti syndrome.

Sixty-nine children attended the follow up clinic at 1 month. Their illnesses and rashes had fully resolved after a variable time period from 1 day to 3 weeks. However eczema was present at follow up in 12 (17%) cases, only three of whom had had a previous history of eczema.

Discussion

An infectious agent was identified in 65 children: 72% were viruses, 20% were bacteria, 5% were *Mycoplasma pneumoniae* and both viruses and bacteria were detected in 3%. All the investigations suggested a current or recent infection. It was of interest that in those children in whom a second serum sample was obtained it did not yield any further information. Whether the infectious agents found were the direct cause of the rash in all cases is unclear. However, the study has highlighted a number of important points.

At presentation, the history, appearance of the rash and associated clinical features were helpful in establishing a clinical diagnosis. The only exception was Gianotti–Crosti syndrome, which it is important to recognize because of its association with hepatitis B in a number of cases.[7] Our two patients were both hepatitis B surface-antigen negative.

Our study has shown that not all 'presumed viral illnesses' are caused by viruses. Other infectious agents identified included Group A β-haemolytic *Streptococcus*, *Mycoplasma pneumoniae*, Group G *Streptococcus* and *Staphylococcus aureus*. Group G *Streptococcus* has been isolated from skin lesions and has caused outbreaks of skin sepsis in hospitals[8] but has not been previously associated with a rash. In the child with *S. aureus* the significance of the enterotoxin C is uncertain as no previous skin rash has been described in association with this enterotoxin. Eleven children had Group A β-haemolytic *Streptococcus* infection which masqueraded as a variety of different rashes. Carriage of Group A β-haemolytic *Streptococcus* may occur in up to 20% of children and is particularly common in crowded communities. However, the patients had a moderate to heavy growth of the *Streptococcus* on throat swab, which is more likely to be associated with infection than asymptomatic carriage[9,10] particularly as the ASO titres were greater than 1 in 320 in all cases.[11] *Streptococcal* M types 3 and 4 (and to a lesser extent type 12) are associated with cases of scarlet fever.[12] Examination of the pharynx was unhelpful as many of the study children had sore red throats. A throat swab is therefore mandatory as it is essential to identify and treat Group A β-haemolytic *Streptococcus* infections promptly with penicillin or erythromycin to prevent the acute complications and reduce the serious risk of glomerulonephritis and rheumatic fever.[13]

Measles was identified in 12 children in whom this had not been recognized clinically. These 12 children did not have Koplik spots and presented with six different patterns of rash, in contrast to most textbook descriptions of a 'morbilliform eruption'. HHV6 has been reported in children with roseola infantum (exanthem subitum).[14] None of the patients proved positive for recent infection with this virus, which was unexpected, but the numbers investigated were small due to the limited availability of this test at the time of our study. HHV6 infection has been reported to cause a mononucleosis-like illness or an acute self-limiting hepatitis.[15]

The majority of the children (85%) were aged less than 5 years old and 43% were less than 1 year. Enteroviral infections were seen most commonly in the younger age group.[2] The association of respiratory syncytial virus infection and a rash in young children is well documented.[16] Four out of twelve children (33%) with measles and two out of seven children (29%) with rubella were under the age of 1 year. Currently MMR vaccination is offered to all children in the U.K. during the second year,[17] usually around 14 months of age. With mass vaccination, a profound effect on the epidemiology of measles, mumps and rubella is expected.[18]

The recovery of infectious agents was 25% greater in males. A male sex predilection has been found in previous studies of both viral and bacterial infections[19,20] and in one study there was a 50% greater recovery of infectious agents in males than in females.[21] The reason for this sex difference is unknown.

It was noted that certain viral infections were seasonal. The incidence of measles peaked in winter and picornaviruses peaked in the spring and summer months.[1] All the other infections were scattered throughout the year.

The two children with combined Group A β-haemolytic *Streptococcus* and enteroviral infections and the child with Kawasaki's disease and Group A β-haemolytic *Streptococcus* are of interest. In these children the cause of the rash is unclear.

Another unexpected aspect of this study was the subsequent development of eczema in 12 out of the 69 children (17%) seen at follow up, within 4 weeks of their illness. Only three of these children had had a previous history of eczema. The age range of nine children who developed eczema for the first time was 3 months–5 years, with a mean age of 1·5 years. These children had a variety of infections: echovirus in three, rubella in two, measles in one, *Mycoplasma pneumoniae* in one and adenovirus in one child. The onset and exacerbation of atopic eczema triggered by systemic infections has been previously reported.[22]

The child with an acute febrile illness and rash is a common paediatric problem. We recommend that routine management should include a throat swab for bacterial investigation. In selected cases in whom the establishment of a diagnosis is important (where there is a risk to a pregnant woman or to immunocompromized individuals) investigations should include a blood screen for rubella, measles, hepatitis A and B, Epstein–Barr virus, parvovirus and *Mycoplasma pneumoniae*.

Acknowledgments

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References

1 Cherry JD. Newer viral exanthems. *Adv Pediatr* 1969; **16**: 233–86.

2 Wenner HA. Virus disease associated with cutaneous eruptions. *Progr Med Virol* 1973; **16**: 269–36.

3 Department of Community Medicine, Tower Hamlets Health Authority, Tower Hamlets People. Health Report. 1988.

4 Department of Community Medicine, The City and Hackney Health Authority. Health in Hackney Annual Report. 1987.

5 Lenette EH, Schmidt NJ (eds). *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections*. 5th edn. Washington: American Public Health Association, 1979.

6 Llanes-Rodas R, Liu C. Rapid diagnosis of measles from urinary sediments stained with fluorescent antibody. *N Engl Med* 1966, **275**: 516.

7 Gianotti F. Papular acrodermatitis of childhood. An Australia antigen disease. *Arch Dis Child* 1973; **48**: 794–99.

8 Efstratiou A. Outbreaks of human infection caused by pyogenic streptococci of Lancefield groups C and G. *J Med Microbiol* 1989; **29**: 207–19.

9 Haverkorn MJ, Valkenburg HA, Goslings WR. Streptococcal pharyngitis in the general population. 1. A control study of Streptococcal pharyngitis and its complications in the Netherlands. *J Infect Dis* 1971, **124**: 339–47.

10 Breese BB, Disney FA, Talpey WB, Green JL. Beta-haemolytic streptococcal infection. The clinical and epidemiological importance of the number of organisms found in cultures. *Am J Dis Child* 1970; **119**: 18–26.

11 Rantz LA, Maroney M, DiCaprio JM. Antistreptolysin O response following haemolytic streptococcal infection in early childhood. *Arch Intern Med* 1951; **87**: 360–71.

11 Gaworzewska E, Colman G. Change in the pattern of infection caused by *Streptococcus pyogenes*. *Epidemiol Infect* 1988, **100**: 257–69.

13 Dobson SRM. Group A streptococci revisited. *Arch Dis Child* 1989; **64**: 977–80.

14 Yamanishi K, Okuno T, Shiraki K *et al*. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. *Lancet* 1988; **i**: 1065–7

15 Irving WL, Cunningham AL. Serological diagnosis of infection with human herpesvirus type 6 *Br Med J* 1990; **300**: 156–9.

16 Berkovich S, Kibrick S. Exanthem associated with respiratory syncytial virus. *J Pediatr* 1964; **65**: 368–70.

17 Department of Health and Social Security. *Immunisation against Infectious Disease*. London: HMSO, 1988.

18 Morgan-Capner P, Wright J, Miller CL, Miller E. Surveillance of antibody to measles, mumps and rubella by age. *Br Med J* 1988; **297**: 770–2.

19 Washburn TC, Medearis DN Jr, Childs B. Sex differences in susceptibility to infections. *Pediatrics* 1965; **35**: 57–64.

20 Lerner AM, Klein JO, Cherry JD, Finland M. New viral exanthems. *N Engl J Med* 1963; **269**: 678–85.

21 Cherry JD, Jahan CL. Virologic studies of exanthems. *J Pediatr* 1966; **68**: 204–14.

22 Harper J, Staughton R, Byrom NA. Atopic eczema after viral infection. *Br Med J* 1982; **284**: 117.

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Are psoriatic patients at risk of heat intolerance?

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Summary

Sixteen young male subjects with psoriasis (mean of 4·9% of skin surface area involvement) and 10 healthy controls underwent a heat exercise test (40°C, 40% r.h.) for 2 h. Rectal temperature (Tr), mean skin temperature (Tsk), heart rate (HR) and heat storage (dS) were measured and calculated. A sharper rise was found for all parameters in the psoriatic patients as compared with controls. Statistically significant differences were found in Tr after 60 min (37·9 ± 0·1°C and 37·5 ± 0·1°C in patients and controls, respectively) and at termination of the exercise (38·3 ± 0·1°C and 37·5 ± 0·1°C). Heat storage at the end of the first hour was 78 ± 9 and 30 ± 7 kcal in patients and controls, respectively. At the end of 120 min, heat storage in the study group increased to 87 ± 14 kcal, while the control group stored only 30 ± 7 kcal. Sweat rate was lower in the psoriatic patients (590 ± 49 g/h) than in controls (691 ± 42 g/h), even when corrected for healthy skin area (337 ± 26 g/h/m^2 compared with 370 ± 24 g/h/m^2). It is suggested that psoriatic patients have a reduced ability to dissipate extra heat during exposure to exercise in the heat. Psoriasis should therefore be considered as a risk factor for heat intolerance.

Heat intolerance is a syndrome in which the body cannot dissipate heat efficiently. Thus under heat stress conditions a rapid increase in body temperature occurs. This predisposes the subject to heat illness and multisystem damage.[1,2] The aetiology of heat intolerance is poorly understood but some congenital, functional and acquired risk factors have been associated with decreased heat tolerance.[3] The identification of heat-intolerant subjects is important in order to prevent the risk of heat injury in physically active persons in hot climates. Previously, Epstein[3] suggested a diagnostic test in which a state of heat intolerance is determined when rectal temperatures of 38·0°C and 38·5°C are reached after 1 and 2 h of a standard exercise heat test.[3]

The main mechanism of dissipating extra heat in a warm climate is by sweat secretion and its evaporation.[4] The full integrity of the skin and its appendages is of importance for the efficiency of this mechanism.[5] Previously it has been shown that extensive damage to the skin or malfunction of its appendages, as in healed burns, miliaria rubra, and congenital ectodermal dysplasia, may adversely affect thermoequilibrium.[5–7] The possible deleterious influence on heat intolerance of other more common diseases affecting the skin has not been assessed. Psoriasis is a very common disease, with an incidence of 1–2% in the general population.[8] Patients with widespread psoriasis may have a decreased healthy body surface area capable of secreting and evaporating sweat. This may compromise thermoregulation, leading to excessive heat accumulation during physical training. The aim of this study was to investigate whether psoriatic patients are more at risk of heat intolerance.

Methods

Subjects

Sixteen young males were included in the study group with varying degrees of involvement of their skin with psoriasis. Ten young healthy male volunteers with physical characteristics similar to the study group (Table 1) served as controls.

Protocol

All the subjects were examined while walking for 2 h on a treadmill (Quinton Q65) at a pace of 5 km/h and a slope of 1% (moderate exercise of 80 W) under controlled environmental conditions of 40°C and 40% r.h. During exposure to heat, rectal and skin temperatures were measured using thermistor probes (YSI 40 and YSI 409, respectively) with a Tele-thermometer (TUC 46). The rectal temperature (Tr) was recorded 10 cm beyond the

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Table 1. Patient and control data

	Study group	Control group
Number of subjects	16	10
Age (years)	20·4±0·2	21·0±0·3
Height (cm)	177±1	176±2
Weight (kg)	72·32±1·76	66·73±2·68
Surface area (m²)	1·89±0·03	1·87±0 02

Values are means±standard error.

anal sphincter. The skin temperature (Tsk) was recorded on the arm, chest and thigh on apparently normal skin. The weighted skin area was calculated according to Ramanatan.[9] The heart rate (HR) was monitored by telemetry (Nihon Koidan Life Scope 6) every 15 min. The sweat was measured by the weight difference at hourly intervals on a scale accurate to ±10 g, corrected for water intake and urine output. Metabolic and respiratory weight loss and oxidative water formation were not considered in the calculation of the sweat rate because they constituted a relatively minute fraction of total weight loss in this study. The criteria for terminating the test were a heart rate above 170 beats per min (b.p.m.), Tr above 39·0°C or subjective complaint of dizziness and exhaustion. The protocol for this study was reviewed and approved by the Institutions Ethical Committee of Investigations Involving Human Subjects.

Estimation of psoriatic skin area

The subjects' body surface areas were estimated according to Dubois and Dubois.[10] The diseased skin area was measured as described by Shapiro *et al.*[11] The affected skin was covered by a translucent polyethylene sheet of uniform thickness and the boundaries of the psoriatic plaques were marked and cut out of the sheet. The polyethylene pieces were weighed on a semi-analytical scale. A calibration curve (area–weight) was plotted using polyethylene squares of known area. The surface areas of the psoriatic plaques were then calculated from the calibration curve. Finally, the percentage of affected area relative total body surface was calculated.

Statistics

The data was statistically analysed with polynomial transformation by repeated measures analysis of variance. The results of each group at 15 min intervals were compared by Tukey's studentized range test (confidence level of 95%). The results are presented as mean±SEM.

Results

The anthropometrical characteristics of the subjects who participated in the study are shown in Table 1. No significant differences were found between the two groups.

The rectal temperatures in the study group increased over the entire 2 h exercise test period to a peak of 38·3±0·1°C (Fig. 1). In the control group Tr reached its peak (37·5°C±0·1) after 45 min and then remained steady. There was a significant difference between the two groups after 60 min.

During the first hour, the skin temperatures of the control group were significantly higher than those of the study group (Fig. 2). This difference disappeared after 60 min due to a greater increase in Tsk among the psoriatic patients. Whereas Tsk in the study group rose continuously to a peak of 36·5±0·2°C at the end of the 2 h period, Tsk of the controls reached a peak of 36·8±0·2°C after 60 min and then decreased to 36·3±0·2°C at the end of the test. At the end of the exercise, the heart rates were 137±7 and 120±6 b.p.m. in the study and control groups, respectively (Fig. 3).

The mean heat storage for both groups is shown in Figure 4. The psoriatic patients stored heat at a higher rate than the control group with a significant difference after 60 min. After 2 h the heat storage of the patients reached 87±14 kcal, while that of the control group was only 30±7 kcal.

The mean total body sweat volumes are shown in Figure 5. These volumes (590±99 and 690±84 ml/h

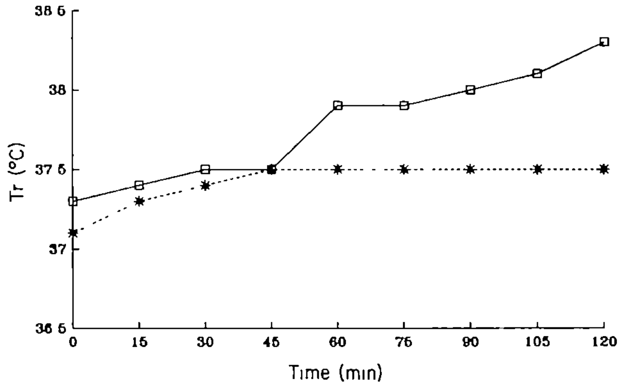

Figure 1. Changes in rectal temperature (°C) in subjects during 2 h of exercise in the heat. □, psoriasis; *, control.

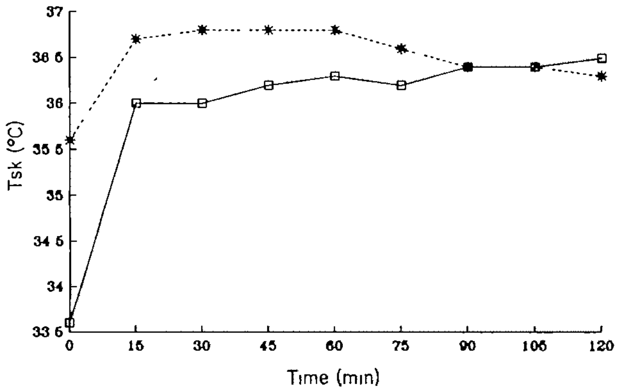

Figure 2. Changes in skin temperature (°C) in subjects during 2 h of exercise in the heat. □, psoriasis; *, control.

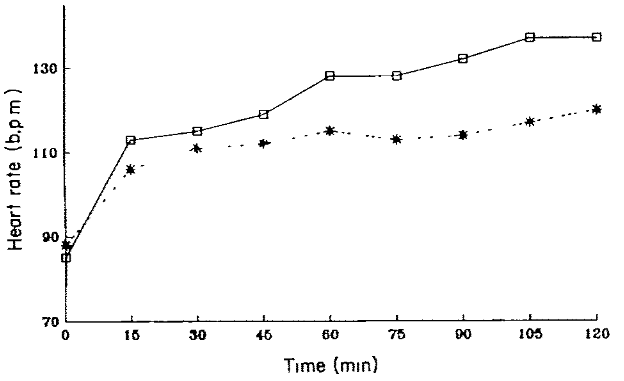

Figure 3. Changes in heart rate (b.p.m.) in subjects during 2 h of exercise in the heat. □, psoriasis; *, control.

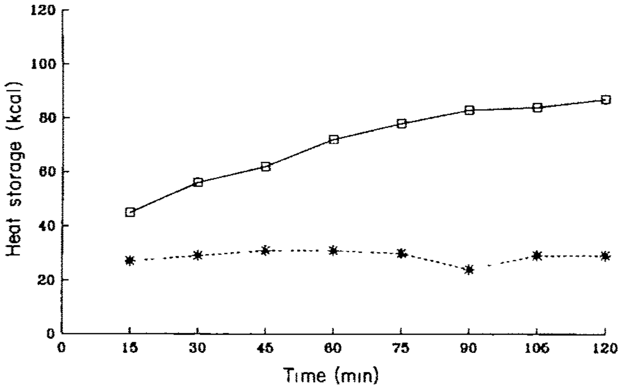

Figure 4. Heat accumulation (kcal) in subjects during exposure to exercise in the heat. □, psoriasis; *, control.

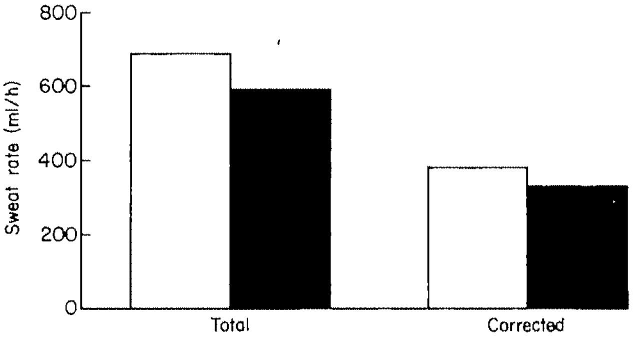

Figure 5. Sweat rate in subjects during 2 h of exposure to exercise in the heat, expressed as total sweat rate (ml) per hour (left) and corrected for normal skin (right) ■, psoriasis; □, control.

Table 2. Thermoregulatory parameters after 60 and 120 minutes of exercise

	Time (min)			
	60		120	
Group	Study	Control	Study	Control
Tr (°C)	37·9±0·1	37·5±0 1	38·3±0·1	37·5±0·1
Tsk (°C)	36·3±0·2	36·8±0 2	36·5±0·2	36·3±0·2
HR (b.p.m.)	128±4	115±4	137±7	120±6
dS (kcal)	72±7	31±6	87±14	29±9

Values are means±standard error of rectal temperature (Tr), mean weighted skin temperature (Tsk), heart rate (HR) and heat storage (dS).

for patients and healthy subjects, respectively), were proportional to the amount of normally functioning healthy skin. However, even after correcting the sweat rate to healthy skin surface area (1·80±0·03 m^2 in the study group compared with 1·87±0·02 m^2 in the controls), it remained higher in the control group (379±49 ml/h) than in the psoriatic patients (328±53 ml/h). The various thermoregulatory parameters after 60 and 120 min of exercise are summarized in Table 2.

Discussion

This study indicates that patients with psoriasis tolerate heat to a lesser degree than normal controls as shown by an excessive rise in rectal temperature and an accumulation of heat.

Differences in heat storage may result either from excessive heat accumulation or reduced ability to dissipate it. The amount of heat required to be dissipated, which is a function of metabolic heat production and temperature gradient between the skin and the environment, was similar in both groups.

Psoriatic patients have a decreased capacity for sweat secretion. The main cause is mechanical occlusion of the sweat ducts by keratin and removal of this blockage allows higher rates of sweating.[12,13] Sweat pore occlusion also occurs in the clinically normal skin of psoriatic subjects but with lesser frequency.[14] The impairment of sweat gland activity may thus be more extensive than the apparent area of psoriatic skin, as shown by the decreased sweat rates found in our subjects even after correcting for healthy skin surface area.

Evaporation of sweat depends upon dilatation of dermal capillaries and increasing skin perfusion. Heat moves by convection from the body core, where it is generated, to the periphery where it is used to evaporate sweat.[15] Psoriatic plaques isolate the skin surface, so that sweat can not be effectively evaporated. The capillaries of psoriatic patients may lack the ability to constrict in a cold environment or dilate in the heat. Evidence of this malfunction was provided when the fingertips of the hands of patients with active psoriasis were immersed in ice water and remained at higher temperatures than those of controls.[16] Similar disturbances in peripheral perfusion were seen in subjects with artificially induced miliaria rubra who were heat intolerant.[6]

Subjects with burns covering less than 30% of body surface were found to have a reasonable tolerance to work in heat by increased compensatory excretion of sweat through normal skin.[17] Patients with burns covering more than 40% of body surface area showed heat intolerance, because the excess sweat was only partially evaporated and the process was relatively inefficient.[5,11] Psoriatic patients, however, were not found to have increased sweat secretion rates when compared with normal controls. It is suggested that the thermoregulatory mechanisms of the burned patients are constantly affected by the large damaged areas. This induces the development of the compensatory mechanism of a relatively increased sweating rate of the healthy skin. The psoriatic patients, with a relatively small diseased skin area (4·9% of total body surface) were shown to have nearly normal thermoregulatory responses under moderate environmental conditions. Only exposure to extreme conditions such as in the present study, has revealed that they have a reduced ability to dissipate the extra heat generated during physical effort. Because they accumulate heat, psoriatic patients could be considered under certain conditions as being heat intolerant.

We conclude that psoriasis is a risk factor for heat intolerance. This is of importance because a common therapy in psoriasis is exposure of the skin to sunlight[16] and many patients may live in hot climates exposed to extreme environmental conditions, where they may undertake various degrees of physical exercise. Such subjects must be aware of the need to be carefully monitored before and during exercise.

References

1 Shapiro Y, Seidman DS. Field and clinical observations of exertional heat stroke patients. *Med Sci Sports Exerc* 1990; 22: 6–14.
2 Knochel JP. Heat stroke and related heat stress disorders. *Dis Month* 1989; 35: 301–78.
3 Epstein Y. Heat intolerance: predisposing factor or residual injury. *Med Sci Sports Exerc* 1990; 22: 29–35.
4 Shapiro Y, Pandolf KB, Goldman RF. Predicting sweat loss response to exercise, environment and clothing. *Eur J Appl Physiol* 1982; 48: 83–96
5 Ben Simchon C, Tsur H, Keren G *et al.* Heat tolerance in patients with extensive healed burns. *Plast Reconstr Surg* 1981, 67: 499–504.
6 Pandolf KB, Griffin TB, Munro EH, Goldman RF. Heat intolerance as a function of percent of body surface involved with miliaria rubra. *Am J Physiol* 1980; 239: R233–R240.
7 Shoenfeld Y, Shapiro Y, Fisher BK, Dvoretsky I. Sweating in the anhidrotic type of congenital ectodermal dysplasia. *Dermatologica* 1975; 150: 277–82.
8 Farber EM, Van Scott EJ. Psoriasis. In: *Dermatology in General Medicine* (Fitzpatrick TB, Eisem AZ, Wolff K, Freedberg IM, Austen KF, eds). New York: McGraw-Hill 1979; 233–47.
9 Ramanatan NL A new weighing system for mean surface temperature of the human body *J Appl Physiol* 1964; 19: 531–3.
10 Dubois D, Dubois EF. Clinical Colometry. A formula to estimate the approximate surface area if height and weight are known. *Arch Int Med* 1916; 17: 863.
11 Shapiro Y, Epstein Y, Ben Simchon C, Tsur H. Thermoregulatory response of patients with extensive healed burns. *J Appl Physiol* 1982, 53: 1019–22
12 Shuster S, Johnson C. The abnormality of sweat duct function in psoriasis. *Br J Dermatol* 1969; 81: 846–50.
13 Johnson C, Shuster S. Eccrine sweating in psoriasis. *Br J Dermatol* 1969; 81: 119–23.
14 Dawber RPR. Psoriasis and adnexal structures. *Br J Dermatol* 1981; 105: 357–9.
15 Astrand PO, Rodahl K. *Textbook of Work Physiology*. New York: McGraw-Hill, 1979; 525–57.
16 Warshaw TG. Psoriasis and thermoregulation. *Int J Dermatol* 1983; 22: 518–19.
17 Epstein Y, Shapiro Y, Brill S. Role of surface area to mass ratio and work efficiency in heat intolerance. *J Appl Physiol* 1983; 54: 831–6.

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The action spectrum between 320 and 400 nm for clearance of psoriasis by psoralen photochemotherapy

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Summary

We have compared the effectiveness of oral 8-methoxypsoralen photochemotherapy (PUVA) using ultraviolet fluorescent lamps with peak emission at either 325, 352 or 370 nm in the treatment of 24 patients with psoriasis. The forearms of each patient were treated three times weekly with two of the three lamps. The erythemal sensitivity of each patient was tested before the first treatment to ensure that equally erythemal doses of radiation were given from each of the lamps. A side-to-side comparison was used to assess response to treatment at weekly intervals for the 6 weeks of the trial. The lamp with peak emission at 325 nm was shown to be significantly superior to either of the other lamps in terms of response assessed at weekly intervals, and time to clearance of psoriasis. An action spectrum, constructed from the median doses required for clearance of psoriasis using each of the lamps, showed that the effectiveness of the radiation decreased exponentially with increasing wavelength throughout the UVA waveband, such that radiation at 320 nm was an order of magnitude more effective than at 360 nm. This suggests that lamps with peak emission around 325 nm will be more effective than those conventionally used in PUVA units with a peak emission at 352 nm. Lamps with peak emission around 325 nm are also highly effective in the treatment of psoriasis with phototherapy alone. Thus a single treatment unit containing these lamps could be used either for PUVA or ultraviolet phototherapy of psoriasis, avoiding duplication or irradiation equipment.

The radiation sources used in psoralen photochemotherapy of psoriasis (PUVA) are almost invariably UVA fluorescent lamps with a fluorescence spectrum extending from about 320 to 400 nm, with a peak at around 352 nm. The choice of this particular spectral power distribution was based not on knowledge of the therapeutic action spectrum, but because in the early 1970s when the treatment of psoriasis by PUVA was introduced, availability of high intensity, wide area UVA sources was limited to lamps with this spectrum.

Research on the action spectrum for healing of psoriasis by ultraviolet radiation alone[1] has led to the development of lamps with emission spectra tailored to the therapeutic action spectrum. These new lamps are said to be clinically more effective than those used conventionally in the treatment of psoriasis.[2–4] The action spectrum for clearance of psoriasis by psoralen photochemotherapy is unknown, but if it does not correspond to the action spectrum for PUVA erythema, development of more effective lamps which exploit this difference may be possible.

Action spectroscopy, in most photobiological research, involves the use of an irradiation monochromator to deliver a narrow waveband of radiation, Technical factors limit the irradiation field with this technique to less than about 10 cm^2. This small field size presents problems when treating lesions of psoriasis over several weeks. An alternative technique, and the one adopted here, is to use a number of large area lamps with different emission spectra and to deduce the therapeutic action spectrum by a process of induction.

Methods

Ultraviolet radiation sources and dosimetry

Fifteen 60 cm fluorescent lamps were mounted in a cylindrical configuration (27-cm diameter) within three identical housings (Fig. 1), allowing uniform irradiation of a patient's hand and arm placed along the central axis into the 'cylinder' of radiation. Each lamp housing was fitted with commercially available fluorescent lamps with peak emission (Fig. 2) at either 325 nm (Wolff Helarium B1-01-40W; Kosmedico, Stuttgart, Germany), 352 nm (TL 40W/09R; Philips, Eindhoven, The Nether-

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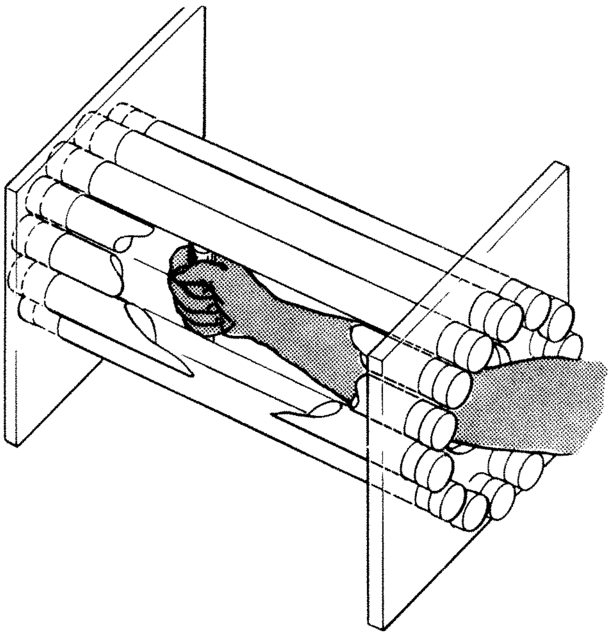

Figure 1. The cylindrical irradiation chamber housing 15 fluorescent lamps. The line drawing omits the protective outer casing and demonstrates the position of a hand grip.

lands) or 370 nm (TL 40W/10R; Philips). These lamps are subsequently referred to as 325-, 352- and 370-nm lamps, respectively. An ultraviolet photodiode, mounted inside each lamp housing, was connected to a specially designed 'dose computer' which controlled the exposure time depending upon the pre-set dose and ultraviolet irradiance. The photodiode in each lamp housing was calibrated with a spectroradiometer (model 742; Optronic Labs Inc, Orlando, FL, U.S.A.) and statements of ultraviolet irradiance and dose refer to unweighted values.

Patients and irradiation protocol

We studied 24 patients (16 female, eight male; age range 18–54 years) who were due to receive whole-body PUVA treatment for psoriasis. The only requirement for entry into the trial was that both forearms were affected by psoriasis to approximately the same extent.

Patients were allocated randomly (in balanced blocks of six) to be treated with two of the fluorescent lamp units (one for each arm). All exposures took place 2 h after ingestion of 8-methoxypsoralen (0·6 mg/kg). On the patients' first attendance a 1-cm-diameter site on uninvolved skin of the inner aspect of each forearm was exposed to a single dose of radiation from the appropriate lamps (Table 1). At 72 h after exposure the intensity of erythema at each site was measured using a reflectance instrument[5] and the increase in erythema index over background levels calculated.[6] Based on this value and the known slope of the PUVA erythema dose–response curve for each of the lamp types[7] (P.M. Farr and B.L. Diffey, unpublished data), the starting dose of UVA was chosen individually for each patient, and was designed to be 40% of the minimal phototoxic dose (Table 1). Treatment was given three times weekly (Monday, Wednesday, Friday) for 6 weeks and doses were increased by 40% after each block of three treatments (1 week).

Assessment of response was made by one clinician (E.M.H.) who was unaware of the types of lamp being used in individual patients. Before the trial, and then at weekly intervals, the severity of psoriasis on each patient's forearms was judged as being equal, more severe on one arm than the other, or completely clear. The same clinician noted whether erythema was present. The 40% weekly increment in dose was abandoned

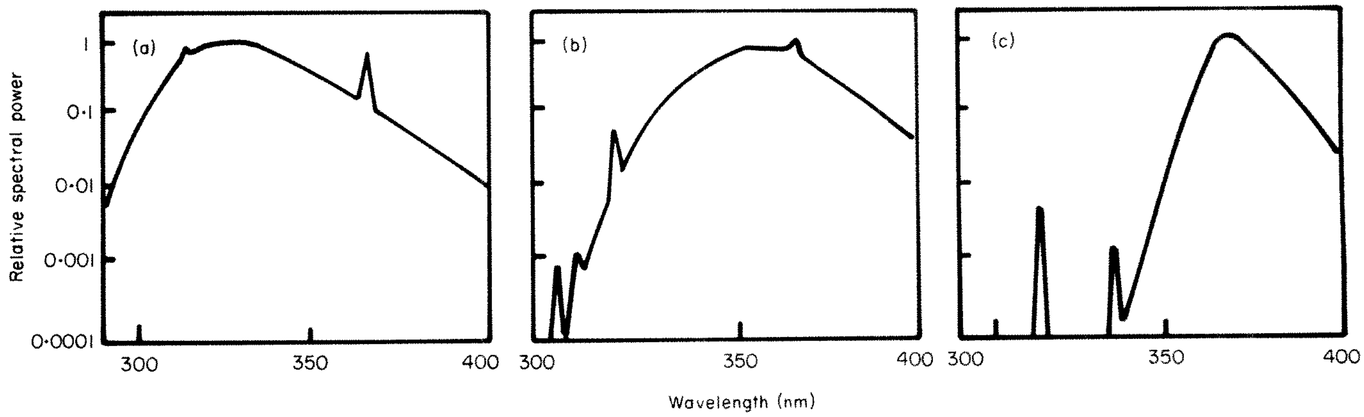

Figure 2. The spectral power distribution of the three ultraviolet fluorescent lamps. (a) 325-nm lamp (Wolff Helarium B1-01-40W); (b) 352-nm lamp (Philips TL40W/09R); (c) 370-nm lamp (Philips TL40W/10R).

Table 1. The starting doses for treatment with each lamp calculated from the increase in erythema index (ΔE) measured 72 h after irradiation of a test site with 2 J/cm^2 (325-nm lamp), 10 J/cm^2 (352-nm lamp) and 30 J/cm^2 (370-nm lamp). (Minimal erythema corresponds to $\Delta E=0{\cdot}05$; moderate erythema $\Delta E=0{\cdot}1$; severe erythema $\Delta E=0{\cdot}2$.[6])

Increase in erythema index 72 h after irradiation	Starting dose (J/cm^2)		
	325-nm lamp	352-nm lamp	370-nm lamp
>0·10	0.7	1·4	2.5
0·09–0·10	0·7	2	3·5
0·08–0·09	0·7	2	3·5
0·07–0·08	1	2·8	5
0·06–0·07	1	2·8	7
0·05–0·06	1	4	10
0·04–0·05	1	4	14
<0·04	1·4	5·6	14

if 'moderate' erythema was present and the next treatment missed if there was 'severe' erythema. On each treatment day, after completion of the arm irradiation, the patients received PUVA therapy to the remainder of the skin using standard whole body UVA units fitted with TL09 lamps. During each treatment the arms were protected from further UVA exposure using tubular bandage, shown to allow negligible (<0·1%) UVA transmission.

Statistical analysis

The analysis compared the effects of treatment on time to clearance of psoriasis for each arm. As some arms did not clear during the study, these times were treated as 'censored data' using the terminology of survival analysis. Each patient provided two times to clearance (one for each arm), and any test of the hypothesis of no treatment effect needs to take this into account. We applied the Cox proportional hazards model, as adapted to paired data.[8] The method ignored those patients in whom either both arms cleared in the same week, or neither arm cleared, because those patients provided no information on treatment differences. On the null hypothesis of no treatment effect it would be equally likely that either arm cleared first. If the log of the odds of clearance with the 325-nm lamp before the 352-nm lamp is a, and for the 352-nm lamp before the 370-nm lamp is b, then under the proposed model the log of the odds for clearance with the 325-nm lamp before the 370-nm lamp is $(a+b)$. This model can be fitted using standard methods.[9]

A second analysis was performed, using a version of McNemar's test to compare the responses throughout the trial in patients receiving treatment with a particular pair of lamps. If the number of occasions on which treatment 1 gave a better result was s and treatment 2 was preferred on t occasions, then the odds in favour of treatment 1 are s/t; confidence intervals can be found in the usual way.[10] Tests of the hypothesis of no treatment difference can be made using standard methods[11] by assuming that when a difference between the arms is noted, it is equally likely to be for either lamp.

Results

All patients completed the trial. Erythema that was severe enough to cause a planned dose increment to be abandoned or to result in a missed treatment was seen on 13 treatment days. Episodes of burning occurred with similar frequency for each of the lamps (325-nm lamps, four episodes; 352-nm lamps, six episodes; 370-nm lamps, three episodes). At the end of the 6 weeks of treatment both arms were clear of psoriasis in 11 patients (46%) and one arm was clear in a further nine patients (38%) (Table 2). One arm was judged to have responded better than the other in 18 patients (75%); in the remaining six patients (25%) both arms were judged to have responded equally (Table 2).

Statistical analysis 1 (time to clearance of psoriasis)

There was a significant difference in time to clearance between lamps ($P=0{\cdot}014$), with the 325-nm lamp having shorter clearance times than either of the other lamp types. There was no significant difference in response ($P=0{\cdot}75$) when treatment with the 352- and 370-nm lamps was compared.

Statistical analysis 2 (treatment differences)

Odds ratios were calculated (Table 3) showing that treatment with the 325-nm lamps was significantly better than either of the other lamp types ($P<0{\cdot}001$ vs. both lamps). There was no significant difference ($P=0{\cdot}57$) in response when treatment with the 352- and 370-nm lamps was compared. As responses on the same patient were likely to be correlated, the analysis may have exaggerated the significance levels ascribed to the treatment differences between lamps. As it is plausible that responses separated more in time are less correlated, the analysis was therefore repeated using only data from weeks 1, 4 and 6, and very similar results were obtained.

Table 2. The clinical response in each patient recorded at weekly intervals during treatment with the three lamps

	Patient number and lamps used															
	(1)		(6)		(9)		(12)		(15)		(17)		(19)		(21)	
Week of Treatment	352	325	352	325	352	325	352	325	352	325	352	325	352	325	352	325
Pre-	=		=		=		=		=		=		=		=	
1	=		−	+	−	+	−	+	+	−	=		=		=	
2	−	+	−	+	N/A		−	+	+	−	−	+	−	+	=	
3	=		−	C	−	+	=		+	−	=		−	+	=	
4	−	+	C	C	−	+	=		C	−	−	+	−	C	=	
5	−	+	C	C	N/A		=		C	C	−	+	−	C	=	
6	−	C	C	C	−	+	=		C	C	−	+	−	C	C	C
Starting dose (J/cm^2)	5·6	1·4	4	1·4	5·6	1·4	1·4	0·5	2	1	4	1·4	2·8	1·4	2 8	0·7
Cumulative dose (J/cm^2)	293	71	53	16	191	48	48	17	26	22	135	48	73	27	53	28
	(2)		(5)		(7)		(8)		(13)		(14)		(16)		(24)	
	352	370	352	370	352	370	352	370	352	370	352	370	352	370	352	370
Pre-	=		=		=		=		=		=		=		=	
1	=		−	+	+	−	+	−	=		+	−	−	+	=	
2	=		−	+	+	−	+	−	−	+	+	−	−	+	+	−
3	=		=		C	−	+	−	=		+	−	−	C	=	
4	=		−	+	C	−	+	−	−	+	=		C	C	−	+
5	+	−	C	C	C	−	C	−	−	C	=		C	C	−	C
6	=		C	C	C	C	C	−	C	C	−	C	C	C	C	C
Starting dose (J/cm^2)	5·6	14	2·8	7	4	5	2	5	2·8	7	2·8	10	2·8	10	2·8	7
Cumulative dose (J/cm^2)	191	477	73	181	33	91	44	260	127	133	143	456	38	92	104	209
	(3)		(4)		(10)		(11)		(18)		(20)		(22)		(23)	
	325	370	325	370	325	370	325	370	325	370	325	370	325	370	325	370
Pre-	=		=		=		=		=		=		=		=	
1	+	−	+	−	−	+	=		+	−	=		+	−	=	
2	+	−	+	−	+	−	+	−	+	−	+	−	−	+	+	−
3	C	−	+	−	+	−	−	+	+	−	C	−	+	−	C	C
4	C	−	+	−	C	−	=		+	−	C	−	=		C	C
5	C	−	+	−	C	−	C	−	+	−	C	−	=		C	C
6	C	−	C	−	C	−	C	C	C	−	C	−	C	C	C	C
Starting dose (J/cm^2)	1·4	14	1·4	10	1·4	5	0·7	10	1·4	10	1·4	14	1·4	14	1·4	14
Cumulative dose (J/cm^2)	19	662	72	258	23	252	16	333	65	507	10	582	35	420	19	166

=, severity of psoriasis equal on both arms; +, psoriasis less severe than on other arm; −, psoriasis more severe than on other arm; C, complete clearance of psoriasis; N/A, results not available.

Table 3. The odds ratios for comparison of treatment with any two of the three lamps calculated for time to clearance (analysis 1) and treatment differences (analysis 2)

Treatment comparison	Odds of shorter time to clearance on second treatment	95% confidence interval	Odds second treatment is better	95% confidence interval
370 vs. 352	0·74	(0·17, 3·57)	1·33	(0·59, 3·09)
352 vs. 325	10·4	(1·06, 102·1)	5·50	(1·87, 22·0)
370 vs. 325	8·2	(0 97, 69·5)	11·0	(3·45, 56 1)

Discussion

We have shown that the effectiveness of PUVA therapy for psoriasis depends upon the emission spectrum of the lamp used for treatment. The lamp with peak emission at 325 nm was more effective than the lamp conventionally used in PUVA units (352 nm), or a lamp with peak emission at a longer wavelength (370 nm). Erythema is the limiting factor in PUVA therapy, and for any comparison of lamps to be valid it is important that equally erythemal doses of radiation are given. We ensured this by testing the sensitivity of each patient with the appropriate lamp, and by basing the starting doses on the measured intensity of erythema at the test site.

Because of the great patient variation in their response to PUVA therapy, the study was designed to allow within-patient comparison of any two of the three lamps under test. This also allowed us to use a simple clinical assessment of response (whether psoriasis on one arm was more or less improved than the other, or completely clear), and meant that poorly validated severity scores or other measures of response were not required.

That equally erythemal effective doses from each of the lamps caused psoriasis to clear at different rates suggests that the therapeutic action spectrum for PUVA is not the same as the action spectrum for PUVA erythema. The doses required to clear psoriasis with each of the lamps can be used to derive an estimate for the action spectrum for clearance of psoriasis with PUVA. The cumulative dose (D) from a given lamp needed to clear psoriasis can be related to the therapeutic action spectrum by the expression

$$D = \int E(\lambda)\, d\lambda \Big/ \int E(\lambda)\rho(\lambda)\, d\lambda \ \mathrm{J/cm^2} \qquad (1)$$

where $E(\lambda)$ is the spectral power at wavelength λ nm, and $\rho(\lambda)$ is the reciprocal of the dose of radiation at this wavelength required to clear psoriasis. The experimental method of the study, using broad-spectrum lamps rather than monochromatic radiation, did not allow us to measure directly the dose required for clearance of psoriasis at each wavelength. Instead we chose to represent the clearance action spectrum $\rho(\lambda)$ by an exponential curve and used a maximum likelihood method[12] to derive its parameter values, together with an approximate 95% confidence interval for these values (Fig. 3). The median doses for clearance of psoriasis (column 3, Table 4) calculated from equation (1) for this choice of ρ agree closely with the observed median doses (column 2, Table 4) derived from the data in Table 2. We chose an exponential function to represent the clearance action spectrum from 320 to 400 nm not on biological grounds, but because our data allowed sensible estimates for $\rho(\lambda)$ only if we use a curve defined by two parameters, and because exploratory analyses with more complicated curves gave similar results at wavelengths greater than 320 nm. There is too little relevant information in our data to attempt estimation of a $\rho(\lambda)$ for wavelengths less than 320 nm. The conclusion of this analysis is that due to the limited number of lamps used in the study it is not possible to derive a good estimate of $\rho(\lambda)$ throughout the ultraviolet spectrum. However, for wavelengths greater than 320 nm our data suggest that the dose for clearance of psoriasis increases in an approximately exponential manner with increasing wavelength.

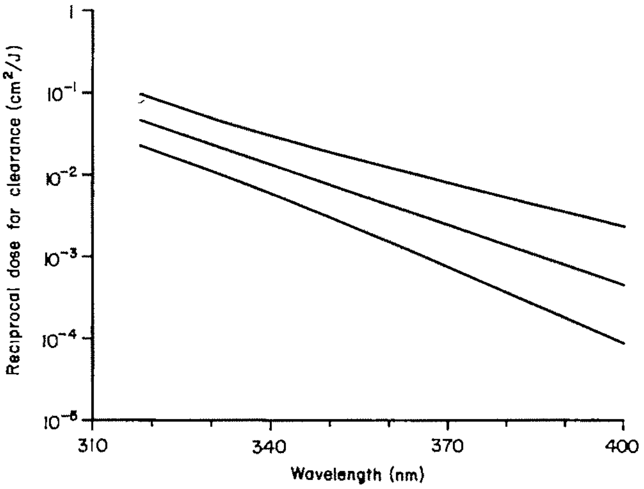

Figure 3. Exponential action spectrum and 95% confidence limits for the clearance of psoriasis by oral 8-methoxypsoralen photochemotherapy.

Phototherapy (without psoralen sensitization) using lamps with peak emission at 325 nm is also effective in clearing psoriasis.[4] With the doses and frequency of irradiation used in this study it is unlikely that the response we observed is independent of the action of psoralen. However, it is possible that the radiation from the 325-nm lamps acted synergistically through both

Table 4. Median UVR doses required for clearance of psoriasis and the clearance doses calculated from equation (1)

Lamp	Median observed clearance dose (J/cm²)	Calculated clearance dose (J/cm²)
325	32	32
352	129	128
370	391	391

psoralen-dependent and psoralen-independent mechanisms.

The results of this study have shown that shortwave UVA radiation (< 340 nm) is more effective than longer wavelength (> 340 nm) radiation in PUVA therapy of psoriasis. A similar wavelength dependence has been shown previously for inhibition of DNA synthesis[13] and induction of sunburn cells in psoralen-sensitized mouse skin.[14] Radiation at wavelengths less than 340 nm, however, comprises only about 16% of the ultraviolet emission from the UVA lamps found in virtually all PUVA irradiation units (typified by the Philips TL09 lamp with peak emission at 352 nm). Lamps such as the TL10 (370 nm) and optically filtered high-pressure mercury lamps (which have the major part of their ultraviolet emission above 360 nm) are increasingly being recommended for PUVA treatment. Our results suggest that much of the UVA radiation from these lamps will be therapeutically ineffective.

As reliable data concerning the wavelength dependence of skin cancer and other hazards of long-term PUVA treatment in man are not available, it is not possible to predict whether the use of 325-nm lamps will result in different risks compared with conventional lamps (352 nm). Preliminary studies in mice treated with 8-methoxypsoralen suggest that the action spectrum for phototumorigenesis is similar to that for erythema.[15] Because the emission spectrum of the 325-nm lamps coincides most closely with our estimate of the action spectrum for PUVA clearance of psoriasis (Fig. 3), and will clear psoriasis with a lower cumulative ultraviolet dose over a shorter time and without a higher risk of acute side-effects than conventional lamps, there is no reason to suppose that any long-term risks will be increased.

We have shown previously that lamps with peak emission around 325 nm are more effective than other sources, such as the 'fluorescent sunlamp' or mercury arc lamp, in combined treatment of psoriasis with ultraviolet phototherapy and dithranol.[4] We have now shown that 325-nm lamps (such as the Helarium B1-01) are more effective for PUVA therapy of psoriasis than ultraviolet lamps with a higher UVA component. The implication of these findings is that either PUVA or ultraviolet phototherapy of psoriasis can be achieved most effectively with the same lamp. Therefore, dermatology units will no longer require separate PUVA and ultraviolet phototherapy equipment.

Acknowledgments

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Note added in proof

The Helarium lamps presently available have a different emission spectrum to those used in this study, and have a peak emission at 340 nm (rather than 325 nm). The therapeutic efficiency of these lamps for PUVA remains to be determined.

References

1 Parrish JA, Jaenicke KF. Action spectrum for phototherapy of psoriasis. *J Invest Dermatol* 1981, **76**: 359–62.

2 van Weelden H, Baart de la Faille H, Young E, van der Leun JC A new development in UVB phototherapy of psoriasis. *Br J Dermatol* 1988; **119**: 11–19.

3 Green C, Ferguson J, Lakshmipathi T, Johnson BE. 311 nm UVB phototherapy—an effective treatment for psoriasis. *Br J Dermatol* 1988; **119**: 691–6.

4 Farr PM, Diffey BL, Marks JM. Phototherapy and anthralin treatment of psoriasis: new lamps for old. *Br Med J* 1987; **294**: 205–7.

5 Diffey BL, Oliver RJ, Farr PM A portable instrument for quantifying erythema induced by ultraviolet radiation. *Br J Dermatol* 1984; **111**: 663–72.

6 Farr PM, Diffey BL. Quantitative studies on cutaneous erythema induced by ultraviolet radiation. *Br J Dermatol* 1984; **111**: 673–82.

7 Cox NH, Farr PM, Diffey BL. A comparison of the dose–response relationship for psoralen–UVA erythema and UVB erythema. *Arch Dermatol* 1989; **125**: 1653–7.

8 Holt JD, Prentice RL. Survival analyses in twin studies and matched pairs experiments. *Biometrika* 1974, **61**: 17–30.

9 McCullagh P, Nelder JA. *Generalized Linear Models*, 2nd edn London: Chapman and Hall, 1989; 98–148

10 Gardner MJ, Altman DG. *Statistics with Confidence*. London. British Medical Journal, 1989; 57.

11 Armitage P, Berry G. *Statistical Methods in Medical Research*. Oxford: Blackwell Scientific Publications, 1987; 115.

12 Aitkin M, Clayton D. The fitting of exponential, Weibull and extreme value distributions to complex censored survival data using GLIM. *Applied Statistics* 1980; **29**: 156–63.

13 Kaidbey K. An action spectrum for 8-methoxypsoralen-sensitized inhibition of DNA synthesis *in vivo* *J Invest Dermatol* 1985; **85**: 98–101.

14 Young AR, Magnus IA. An action spectrum for 8-MOP induced sunburn cells in mammalian epidermis *Br J Dermatol* 1981; **104**: 541-548.

15 Young AR, Walker SL, Garmyn M A first approach to an action spectrum for 8-MOP phototumorigenesis in mice. *J Invest Dermatol* 1988; **90**: 175–8.

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A histopathological study of 643 cutaneous horns

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Summary

A retrospective study of 643 cutaneous horns examined in our department between 1970 and 1989 revealed that 38·9% were derived from malignant or premalignant epidermal lesions, and 61·1% from benign lesions. Four main features were associated with premalignant or malignant histopathological change at the base of a cutaneous horn (base pathology). These were: (i) age—the mean age of the patients whose cutaneous horns showed premalignant or malignant base pathology was 8·9 years greater than the mean age of the patients where base pathology was benign ($P<0{\cdot}0005$); (ii) sex—males were more likely to develop a cutaneous horn with (pre)malignant base pathology ($P<0{\cdot}001$); (iii) site—over 70% of all (pre)malignant lesions were found on the nose, pinnae, backs of hands, scalp, forearms and the face; a cutaneous horn found at these sites was 2·1 times more likely to have derived from a (pre)malignant base than from any other part of the body ($P<0{\cdot}0001$); (iv) geometry of the lesion—lesions with a wide base or a low height-to-base ratio were significantly more likely to show (pre)malignant base pathology. Of those solar keratoses giving rise to cutaneous horns, 8·9% also showed histological evidence of change to squamous cell carcinoma (SCC); this figure was not significantly different from the 6·2% of solar keratoses without horns that showed SCC change during the same period (1970–89).

The description 'cutaneous horn' is the term given to a protrusion from the skin of keratinized material organized in the shape of a horn. There are considerable variations in size and shape, but most are conical. 'Cutaneous horn' is not a pathological diagnosis and the lesion may derive from benign, premalignant or frankly malignant epidermis. The object of this study was to identify factors associated with (pre)malignant base pathology.

Methods

All the cutaneous horns ($n=643$) examined in the Department of Dermatology of the Royal Liverpool Hospital between 1970 and 1989 were reviewed. Clinical details such as the age, sex of patient and the site of the lesion were noted, and the base pathology of each specimen was established according to commonly accepted histological criteria.[1]

The maximum width of the abnormal epithelium of the base and the height of the keratotic mass were measured at a magnification of ×25 using an ocular micrometer. Two groups were distinguished. Group A consisted of horns where the height was at least one-half the width of the base;[2] the remainder were included in Group B. These two groups were compared statistically with regard to age, sex, and base pathology. Cutaneous horns showing at their base the pathology of a solar keratosis were compared with all solar keratoses without cutaneous horns removed during the same period (1970–89). The prevalence of SCC change in solar keratoses in these latter two groups were compared.

Statistical analysis

The data were analysed using the MINITAB® computer software package (Release 6·1, Minitab, Inc.). Where appropriate, the results were expressed in terms of 95% confidence intervals for the parameter or difference in parameters, unpaired Student's t- and χ^2 tests. The measurements of the width of base and height of the horns were positively skewed and logarithmic transformation of the data yielded normalized distributions with approximately 95% of the values lying within two standard deviations.

Results

The 643 cutaneous horns examined represent 1·3% of

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all skin biopsies submitted to our department between 1970 and 1989, an average of 32 new cases per year.

Base pathology

Three-hundred and ninety-three (61·1%) cutaneous horns had benign base pathology. Of these, 150 (23·3%) showed benign epithelial hyperplasia with proliferative basal budding; 135 (21·0%) derived from basal cell papillomas (seborrhoeic warts); 100 (15·6%) derived from viral warts; four (0·6%) from trichilemmal cysts; three (0·5%) from keratoacanthomas; and one from a benign pigmented intradermal naevus. One hundred and forty-nine (23·2%) horns had premalignant base pathology. Of these, 123 (19·1%) derived from solar keratoses; and 26 (4·0%) from Bowen's disease. One hundred and one (15·7%) horns had frankly malignant base pathology. All of these showed changes of SCC, with 12 showing co-existent pathology of solar keratosis.

Site of lesion

One-hundred and fifty-one (23·5%) of all cutaneous horns occurred on the face, the most common site for a cutaneous horn of any pathology. The nose, pinnae, backs of hands and forearms carried a higher than expected chance of finding malignant base pathology (Fig. 1); a similar pattern was found in the premalignant lesions. Seventy-six per cent of all (pre)malignant lesions were found on the nose, pinnae, backs of hands, scalp, forearms and face; a cutaneous horn occurring at one of these sites was 2·1 times more likely to show premalignant or malignant base pathology than one at any other part of the body ($\chi^2=34\cdot3$, $P<0\cdot0001$).

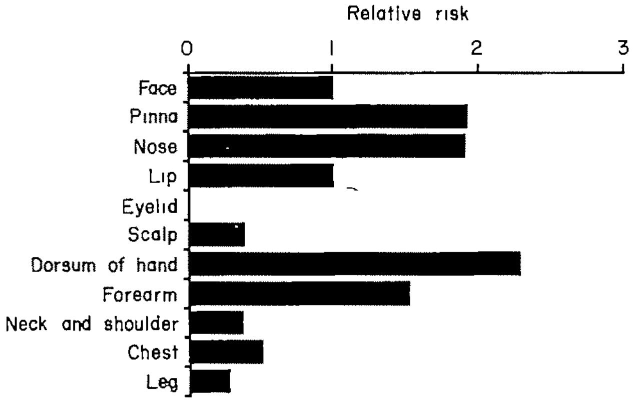

Figure 1. Relative risk of occurrence of malignant base pathology in cutaneous horns according to body site (overall risk = 15·7%).

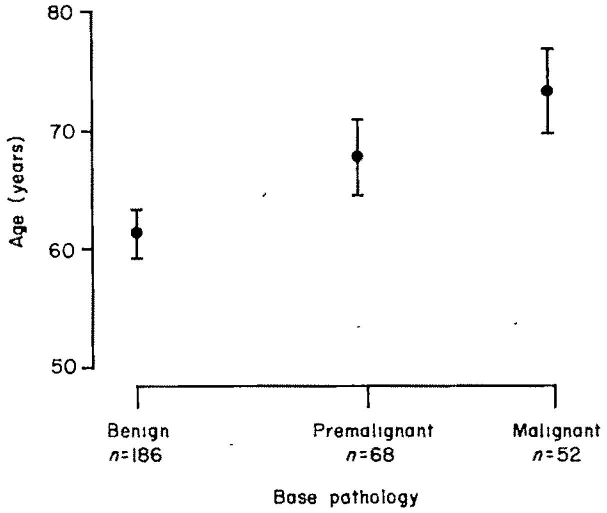

Figure 2. Mean (●) and 95% confidence intervals of patients' age for groups with benign, premalignant and malignant base pathology

Further analysis of Group A

This group conformed to the definition of a cutaneous horn suggested by Bart *et al.*[2] in that the height was at least one-half the width of the base. Using the base pathology and the patients' age and sex as parameters, no significant difference was found between Groups A and B. The two groups can therefore be regarded as a single population.

Age. Patients with a malignant base pathology were on average 5·6 years older than those with premalignant base pathology (95% confidence intervals of the difference in mean age = 0·9–10·2 years, $P=0\cdot02$). Patients in the latter group were on average 6·5 years older than those with benign pathology (95% confidence intervals of the difference in mean age = 2·8–10·3 years, $P=0\cdot0008$). The chance of finding (pre)malignant change in the base of cutaneous horns increased proportionately with age (Fig. 2).

Sex. The overall female-to-male ratio in Group A was 1·17 (180/154). In patients with benign base pathology this ratio was 1·50 (123/82), whereas for those with (pre)malignant base pathology it was 0·79 (57/72). Thus, cutaneous horns with (pre)malignant base pathology were significantly more common in males ($\chi^2=7\cdot97$, $P=0\cdot005$). The sex factor is independent of the age effect as female patients were on average 1·1 years older than the male patients and therefore on the basis of age alone we would have expected to find more females in the (pre)malignant group.

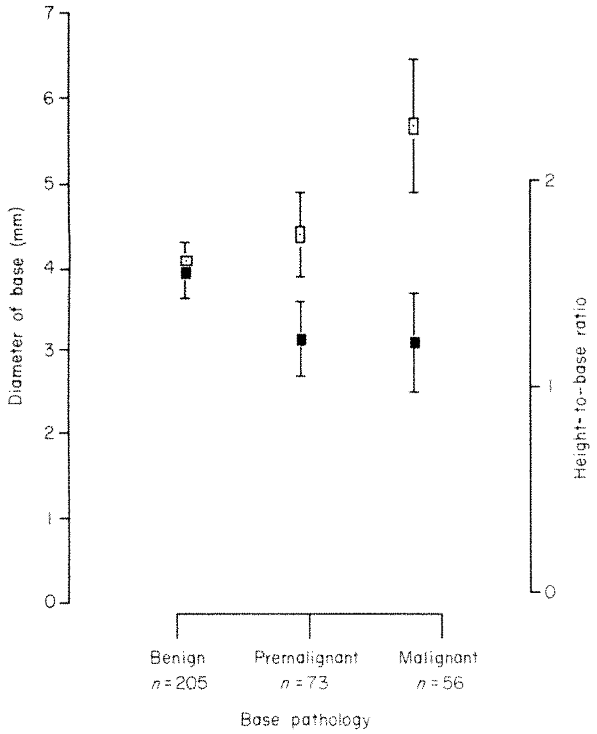

Figure 3. Geometric mean and 95% confidence intervals of base width (□) and height-to-base ratio (■) for groups with benign, premalignant and malignant base pathology.

Geometry of the lesion. The geometric mean base widths of the benign, premalignant, and malignant groups were 4·05, 4·40 and 5·68 mm, respectively. Horns with malignant base pathology had a significantly wider base than either the premalignant ($P=0{\cdot}02$) or the benign lesions ($P<0{\cdot}0001$); there was no significant difference between the benign and the premalignant groups (Fig. 3).

The geometric mean height of the keratotic mass measured 6·25, 5·38 and 6·86 mm in the benign, premalignant and malignant groups, respectively. No significant difference was found between the three groups.

Benign lesions had significantly higher height-to-base ratios than did either the premalignant ($P=0{\cdot}0096$) or the malignant group ($P=0{\cdot}016$). There was no significant difference between the premalignant and malignant groups (Fig. 3).

Co-existent (pre)malignant skin lesions

In the (pre)malignant group 18/250 (7·2%) patients had one or more (pre)malignant skin lesions diagnosed at the time of presenting the cutaneous horns. This was significantly higher than the 6/393 (1·5%) patients found in the group with benign base pathology ($\chi^2=12{\cdot}6$, $P=0{\cdot}0004$). The presence of co-existing (pre)malignant skin lesions increased the likelihood of the cutaneous horn showing (pre)malignant base pathology.

Malignant base pathology

One-hundred and thirty-five cutaneous horns showed the base pathology of a solar keratosis and 12 (8·9%) showed co-existent changes of SCC. During the same period, 2026 solar keratoses without horns were examined histologically and 125 (6·2%) showed co-existent changes of SCC. There was no significant difference in these two groups ($P>0{\cdot}2$) with regard to the incidence of co-existent SCC in solar keratosis.

Discussion

Cutaneous horns are distinguished from other keratotic lesions clinically by the presence of a roughly conical keratotic mass. Bart *et al.*[2] defined a cutaneous horn as a keratotic lesion with a height of at least half the widest diameter of its base. However, specimens are frequently disrupted by trauma during life or at removal. In order to avoid selection bias in a retrospective study, all the specimens labelled as cutaneous horns removed between 1970 and 1989 were included in the study. They were divided into two groups (A and B) on the basis of the height-to-base ratio but there were no statistical differences between the two groups in terms of base pathology, age and sex of patients. The two groups can therefore be regarded as a single population.

Cutaneous horns have been regarded by some as obligatory precancerous or malignant lesions. In a series of 230 cutaneous horns[3] 58% showed either premalignant or malignant changes at their base. Others[2,4] have reported a much higher proportion of horns showing benign histological features. Studies on parameters that are commonly associated with (pre)malignant base pathology have produced conflicting evidence with regard to age and sex of patients, and the shape of the lesion.[2,5] Large cutaneous horns, particularly giant horns, are said to be commonly derived from malignant base.[2,5–7] It is generally agreed that the presence of a keratotic horn does not necessarily imply that a premalignant base pathology, such as a solar keratosis, is more likely to develop frankly malignant change with time. This observation has, however, not been subjected to statistical testing.

Our study confirms that cutaneous horns may arise from a wide range of epidermal lesions which may include benign lesions such as basal cell papillomas, viral warts, keratoacanthomas and trichilemmal cysts, and pre- or frankly malignant lesions such as solar keratoses, Bowen's disease and SCC. The single largest group (23·3%) showed a base pathology of benign epithelial hyperplasia with only proliferative basal budding. It was not possible to determine the precise base pathology in this group. Bart *et al.*[2] suggest that the pathology in this group may represent early basal cell papilloma.

In contrast to the findings of Bart[2] and Mehregan,[5] cutaneous horns were more common in females in our series. However, horns with (pre)malignant base pathology were more common in males. Cutaneous horns with a wide base or a low height-to-base ratio were more likely to show either premalignant or malignant base pathology. The co-existence of other (pre)malignant skin lesions increased the likelihood of finding a horn with (pre)malignant base pathology; but the presence of a horn over a solar keratosis did not increase the likelihood of finding frank malignant change in that solar keratosis.

Acknowledgment

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References

1 Lever WF, Schaumburg-Lever G. Tumours and cysts of the epidermis. *Histopathology of the Skin*, 7th edn, 1990; 523–67.

2 Bart RS, Andrade R, Kopf AW. Cutaneous horns. *Acta Derm Venereol (Stockh)* 1968; **48:** 507–15.

3 Schosser RH, Hodge SJ, Gaba CR, Owen LG. Cutaneous horns: A histopathologic study. *Sth Med J* 1979; **72:** 1129–31.

4 Baruchin A, Sagi A, Lupo L, Hauben DJ. Cutaneous horn (cornu cutaneum). *Int J Tiss Reac* 1984; **6:** 355–7.

5 Mehregan AH. Cutaneous horn: a clinicopathologic study. *Derm Digest* 1965; **4:** 45–54

6 Glickman FS, Frolich S. Giant cutaneous horn. *Cutis* 1982; **30:** 77–8.

7 Semms H, Null HM. Giant cutaneous horn. *JAMA* 1969; **210:** 2285.

British Journal of Dermatology (1991) 124, 453–460. ADONIS 000709639100109G

Abnormal erythemal response and elevated T lymphocyte HRPT mutant frequency in Cockayne's syndrome

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Summary In three children with Cockayne's syndrome (CS), skin exposed to ultraviolet radiation responded transiently either with erythematous papules or an exaggerated sunburn-like response, without chronic actinic damage. Irradiation monochromator tests demonstrated an abnormal delay or reduction in the threshold to ultraviolet (UVB) irradiation-induced erythema similar to that of xeroderma pigmentosum (XP). As with XP there was an elevated frequency of mutants resistant to 6-thioguanine in circulating T lymphocytes. The mutant frequency in a single obligate heterozygote was normal. In contrast to XP, in the two CS individuals studied, adaptive cell-mediated immunity and natural killer cell function were normal. Because the risk of skin cancer is very high in XP but not in CS, the normal immune function in CS provides evidence that immune surveillance may be important in UV tumorigenesis.

Cockayne's syndrome (CS) is a rare autosomal recessive disease characterized by cachetic dwarfism, pigmentary retinal degeneration, mental retardation, progressive demyelinating neurological defects, and acute sun sensitivity.[1–4] The onset of symptoms usually occurs in the first year of life after an apparently normal initial development. Cutaneous photosensitivity is a common presenting feature, yet the clinical time course of skin reactions following sun exposure and *in-vivo* phototesting responses have not been reported.

Photosensitivity in CS is manifest at the cellular level as hypersensitivity to the lethal effects of ultraviolet (UVC) irradiation[5–7] without any gross defects in excision or daughter-strand DNA repair.[6,8] Damage produced in DNA by low doses of UV-light results in only a transient depression of DNA and RNA synthesis in normal cells, while in CS cells recovery does not occur.[8,9] This finding forms the basis of a diagnostic test for the disorder.[10] The demonstration of a specific defect in excision repair of UV damage in actively transcribing DNA in CS[11] may provide an explanation for the lack of recovery of RNA synthesis after UVC treatment.

In xeroderma pigmentosum (XP) defective DNA repair[12,13] results in an increased frequency of induced mutation in cells in culture[14–16] and an elevated mutant frequency to 6-thioguanine resistance in circulating T lymphocytes.[17,18] The condition is also associated with a high rate of cutaneous, and to some extent internal, neoplasia.[19,20] Mutation in circulating T lymphocytes has not been reported in detail in CS, although there is evidence of an elevated mutation frequency following treatment of fibroblasts with UVC.[15,16] There is no evidence in this syndrome for an increased cancer risk and, in contrast to XP, cell-mediated immunity and natural killer cell function are normal.[21]

We report details of cutaneous photosensitivity including *in-vivo* light testing in three children with Cockayne's syndrome. Furthermore, we have shown that in four CS patients the mutant frequency in circulating T lymphocytes was elevated.

Patients and methods

Patient 1

A 2·5-year-old girl presented at age 4 months with acute photosensitivity and exaggerated sunburn of the exposed areas of skin with progression to blistering and delayed resolution. No chronic actinic damage, scarring or cutaneous atrophy was evident.

There was progressive growth retardation from the age of 6 months and by 2·5 years her weight, length and head circumference were well below the third centile. Neurological and developmental assessment at this time revealed marked truncal ataxia, a divergent squint, early

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upper motor neurone signs in the legs, and a global developmental level of around 10–14 months. Her fundi were normal apart from speckling in the periphery.

A CT head scan revealed a very small cerebellum and enlargement of the fourth, third and lateral ventricles. Electroretinographic (ERG) and visual evoked responses (VER) demonstrated moderate impairment of retinal and optic nerve function.

Patient 2

A 14-year-old girl presented at age 2 years with walking difficulties and short stature. Her neurological state continued to deteriorate with increasing ataxia and bilateral sensorineural deafness although her vision has been relatively well preserved despite moderate retinal pigmentation. By 5·2 years of age her height, weight and head circumference were well below the third centile. She had precocious puberty with menarche at 9 years.

Cutaneous photosensitivity, beginning at the age of 4 years, consisted of recurrent eruptions of erythematous papules on the cheeks, forehead and limbs (Fig. 1) several hours following minimal sun exposure and progressing to scaling and desquamation by the next day. Over a period of 4–5 days there was resolution of the lesions without residual scarring. Sun avoidance and regular application of high protection factor sunscreens were advised. Between attacks the skin appeared normal, without loss of subcutaneous fat (Fig. 2).

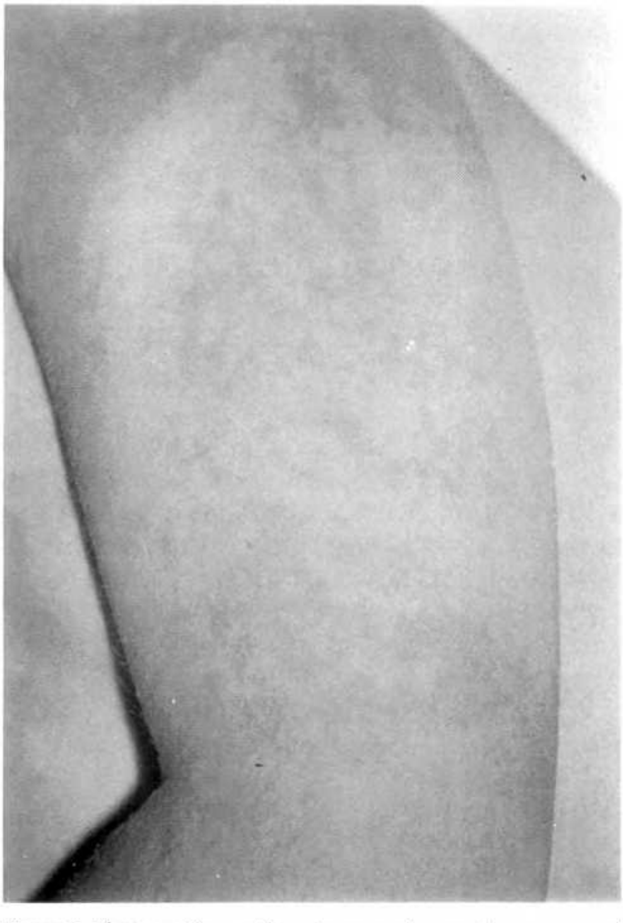

Figure 1. Photosensitive erythematous papular eruption on arm of Patient 2.

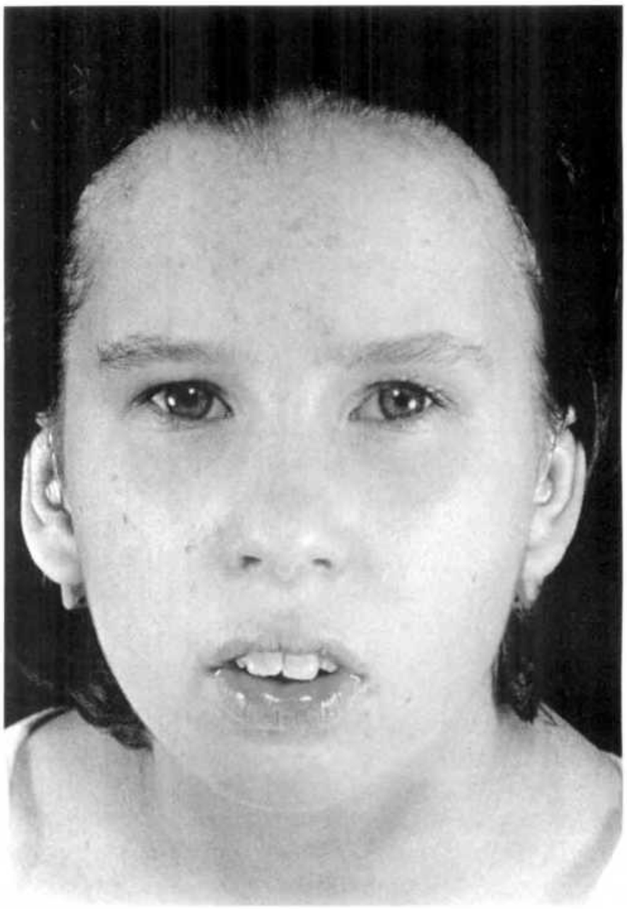

Figure 2. Facial appearance of Patient 2.

A CT scan at the age of 5 showed a widening of the basal subarachnoid space consistent with brainstem atrophy and moderate enlargement of the third and fourth ventricles. ERG and VER studies indicated a marked reduction of retinal function owing to pigmentary degeneration and suggested demyelinating lesions in the optic nerve and possibly optic tract and radiation.

Delayed cutaneous hypersensitivity response of the ventral forearm to seven recall antigens (Multitest CMI, Institut Merieux, Lyon, France) and *in-vitro* lymphocyte transformation responses to 1, 10 and 100 μg/ml of recall antigens streptokinase–streptodornase (SKSD) and tuberculin were normal. The circulating $CD4^+$/

$CD8^+$ cell ratio was 2·0 (normal mean ± SD, 1·89 ± 0·80). Natural killer (NK) cell cytotoxicity measured against the erythromyeloid cell line K562 in a 4-h ^{51}Cr release assay[22] was also normal (specific cytotoxicity 36 at effector:target cell ratio 50:1; normal mean ± SD, 45·8 ± 17·8).

Patient 3

A 14-year-old girl was noted to have microcephaly at age 8 months. By 2 years of age delayed motor development was evident and her height and weight had fallen below the third centile. Her neurological state deteriorated progressively with development of marked ataxia and sensorineural deafness. She is blind and has retinal pigmentation.

Photosensitivity, first noticed at 6 years of age, was characterized by the onset within 1 h following more than a few minutes sun exposure of erythematous papules on exposed skin, particularly the forehead, nose and cheeks. This became increasingly severe over the next 24 h and persisted for about 7 days before resolution without scarring. High protection factor sunscreens had been used regularly from the onset of photosensitivity. She demonstrated sunken facies and generalized hyperpigmentation but no chronic actinic damage.

Immune responses were assessed as for Patient 2. Cutaneous and *in-vitro* recall antigen responses, circulating $CD4^+$:$CD8^+$ cell ratio (1:3) and NK activity (specific cytotoxicity 30 at effector:target ratio 50:1) were all within normal limits.

In-vivo light testing for photosensitivity

The skin of the back of each patient was irradiated with an increasing series of doses of narrow wavebands at 300 nm (dose range 2–200 mJ/cm^2), 307·5 nm (5–500 mJ/cm^2), 320 nm (500–4000 mJ/cm^2), 340 nm (10 J/cm^2), 360 nm (12·5 J/cm^2) and 380 nm (15 J/cm^2) from an irradiation monochromator (Applied Photophysics, London, Model 498/01). The lowest doses to produce erythema and a papular response were recorded at 24, 48 and 72 h after irradiation.

Studies on cultured fibroblasts

A list of the fibroblast cultures used in this investigation, together with the source and donor type is provided in Table 1.

Cell survival. Full details of the methodology for the determination of cell survival have been published elsewhere.[23]

Inhibition of RNA synthesis. Procedures for the investigation of the effect of UVC on post-irradiation RNA synthesis have also been published elsewhere.[10]

Mutant frequency in circulating T lymphocytes

The blood samples used in this study are listed in Table 2. A microtitre cloning technique was used to measure the frequency of circulating 6-thioguanine-resistant mutant lymphocytes.[24,25] Mononuclear cells were separated from heparinized venous blood and cultured for 24 h in

Table 1. Fibroblasts used in this study

Designation	Characteristic	Source
1BR.3	Normal	Cell Mutation Unit, University of Sussex*
GM730		NIGMS†, Camden, U.S.A.
48BR		Cell Mutation Unit, University of Susex*
CS2BR	Cockayne's syndrome	D.Brett,* Great Ormond Street, London
CS4BR		D.Siggers,* Royal United Hospital, Bath
Patient 1	Cockayne's syndrome	P.Botcherby,† Guy's Hospital
Patient 2		P Botcherby,† Guy's Hospital
Patient 3 (CS2BI)‡		A.M R Taylor,† Dept Cancer Studies, Birmingham
Mother of patient 3	Normal	A.M.R.Taylor,† Dept Cancer Studies, Birmingham

*Biopsy or † cells (where biopsy received, fibroblast culture established at CMU by S.A.Harcourt).
‡Assignment by Dr Taylor who has checked cell survival and UDS in this culture.

Table 2. Blood samples used in this study

Designation	Characteristic	Source
Patient 1	Cockayne's syndrome	P.Norris
Patient 2		P.Norris
Patient 3		P.Norris
CSCC		R.MacFaul, Pinderfields General Hospital, Wakefield
Mother of Patient 2	Normal	P.Norris

51 samples from normal non-smokers from birth (cord blood) to age 84 have been included in Figure 5.

RPMI 1640 medium plus 10% human AB serum. The T cells were then cloned in microwells in the presence or absence of 5×10^{-6} M 6-thioguanine in medium containing phytohaemagglutinin, irradiated feeder cells and the T-cell growth factor interleukin 2. Mutant frequencies were compared with the results from normal, non-smoking controls available from our data base using the statistical programme GLIM (Royal Statistical Society, London).[25]

Results

In-vivo light testing for photosensitivity

The appearance of the maximal papular responses followed irradiation with UVB (280–315 nm) and shorter UVA (315–400 nm) wavelengths in all three patients (Table 3). Patient 1 who was clinically most photosensitive, also demonstrated abnormal vesicular responses and a marked reduction of minimal erythema doses at 24 h of ≤2 mJ/cm^2 at 300 nm (normal range 10–20 mJ/cm^2), 10 mJ/cm^2 at 307·5 nm (100–200 mJ/cm^2), ≤500 mJ/cm^2 at 320 nm (>1000 mJ/cm^2) and ≤10,000 mJ/cm^2 at 340 nm (>15,000 mJ/cm^2) (Table 4).

Table 3. Minimal papular doses determined by *in-vivo* irradiation monochromator testing

Wavelength (±half band width) (nm)	Minimal papular dose (mJ/cm^2)								
	Patient 1			Patient 2			Patient 3		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
300 (±5)	≤2	≤2*	NA	NP	200	NP	NP	100	100
307·5 (±2)	20	20	NA	NP	500	NP	NP	200	200
320 (±10)	≤500	≤500†	NA	NP	4000	4000	NP	4000	4000
340 (±10)	NP	NP	NA	NP	NP	NP	NP	NP	NP
360 (±10)	NP	NP	NA	NP	NP	NP	NP	NP	NP
380 (±10)	NP	NP	NA	NP	NP	NP	NP	NP	NP

NA, result not available; NP, no papular response; *, vesicular response at 50 mJ/cm^2; †, vesicular response at 1000 mJ/cm^2.

Table 4. Minimal erythema doses determined by *in-vivo* irradiation monochromator testing

Wavelength (±half band width) (nm)	Minimal erythema dose (mJ/cm^2)									
	Patient 1			Patient 2			Patient 3			Normal range
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h
300 (±5)	≤2	≤2	NA	50	50	50	20	20	20	≥10
307·5 (±2)	20	10	NA	200	200	500	100	100	100	≥100
320 (±10)	≤500	≤500	NA	N	N	N	4000	4000	4000	≥1000
340 (±10)	≤10,000	N	NA	N	N	N	N	N	N	>12,500
360 (±10)	N	N	NA	N	N	N	N	N	N	>15,000
380 (±10)	N	N	NA	N	N	N	N	N	N	>15,000

NA, result not available; N, normal.

Studies on cultured fibroblasts

The response of fibroblasts from three normal and four CS donors to the lethal action of UVC is illustrated in Figure 3. There was no difference in sensitivity between the CS heterozygote (mother of Patient 3) and the three normal cell strains (1BR.3, 48BR and GM730). All four CS cell strains proved unequivocally more sensitive than the normals. Regrettably, the culture from Patient 2 proved to have such a poor capacity for cloning that plating experiments were not possible.

The results of the investigation of post-UV RNA synthesis are summarized in Figure 4(a) and (b). In Figure 4(a) the clear distinction between the response of CS fibroblasts and that from normals or the heterozygote is expressed as a histogram using the data obtained from cells exposed to a UVC dose of 15 J/m^2. Dose–response curves for the inhibition of RNA synthesis are provided for normal (GM730) and two CS cell strains (CS2BR, and Patient 2) in Figure 4(b). They confirm the defective responses of CS fibroblasts.

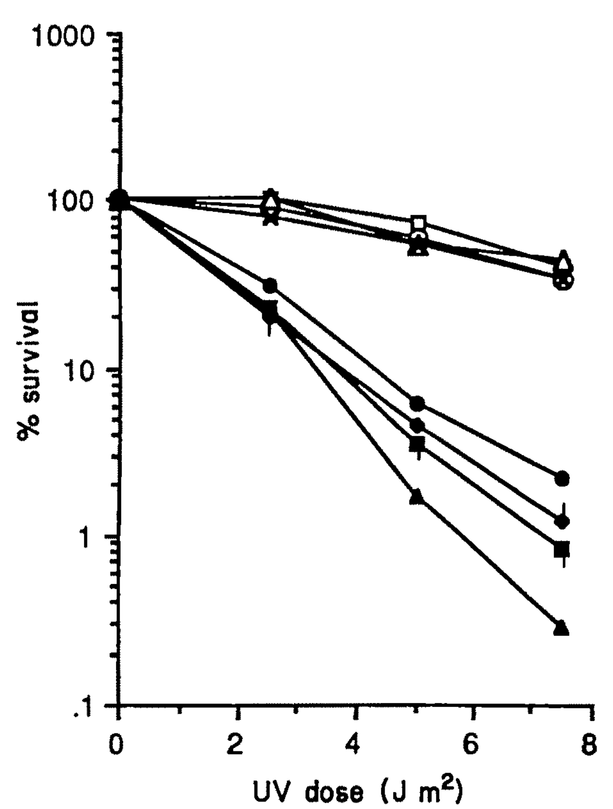

Figure 3. Cell survival in CS cells. Colony forming ability of fibroblasts following irradiation with different doses of UVC. —○—, normal, 1BR.3, mean of five experiments, control cloning efficiency = 53 ± 5%; —□—, normal, GM730, mean of three experiments, control cloning efficiency = 55 ± 0·4%; —△—, normal, 48BR, single experiment, control cloning efficiency = 50%; —▲—, Cockayne's syndrome, CS2BR, single experiment, control cloning efficiency = 65 0%; —●—, Cockayne's syndrome, CS4BR, single experiment, control cloning efficiency = 61 0%; —■—, Cockayne's syndrome, patient 1, two experiments, control cloning efficiency = 91%; —◆—, Cockayne's syndrome, Patient 3, two experiments, control cloning efficiency = 54%; —×—, Heterozygote mother of Patient 3, two experiments, control cloning efficiency = 52% For the controls (1BR.3 and GM730) the standard error of the means is encompassed by the dimensions of the symbols. For the two Cockayne's syndrome cultures with repeat experiments the limits between the two experiments is indicated by a vertical line where this is not obscured by the symbols.

Mutant frequency in circulating T lymphocytes

The mutant frequency for our three patients, one other CS patient, the mother of Patient 2 and our data base for normal non-smoking donors is illustrated in Figure 5 together with the relevant individual results in Table 5. While there was no statistically significant component for CS or the effect of time alone, there was a significant effect for the syndrome × age interaction confirming the elevated (two-fold) mutant frequency for CS. XP patients have a similar raised mutant frequency (2·5-fold, $n = 10$), but in this case the significance was higher ($P < 0{\cdot}001$). The reason for this difference in significance was that only small blood samples could be obtained from the CS donors, and the cells cloned poorly, so that the estimate of the mutant frequency was less satisfactory. Using our method of analysis, it is not possible to make definitive statements about the results from individual donors, but the single heterozygote mother of Patient 2 lies at the top of the range for normal, non-smoking adults.

Discussion

In 1936, Cockayne[1] described two children with dwarfism, retinal atrophy, deafness, and a scaly, erythematous dermatitis that affected the dorsum of the hands, legs, face and ears and which was exacerbated by sun exposure. Later, three similar cases were reported with photosensitive dermatitis limited to exposed sites, particularly the 'butterfly' area of the face.[26] However, in 1959, MacDonald *et al.*[4] described three patients characterized by a tendency to severe sunburning, and associated with pigmentation and atrophic scarring of exposed skin. Patient 1 experienced similar easy sunburning with blistering and delayed resolution of any exposed skin areas, closely resembling that characteristic of many patients with xeroderma pigmentosum.[27] In contrast, Patients 2 and 3 developed an erythematous papular eruption, particularly on the face, the onset being similarly delayed for several hours following sun

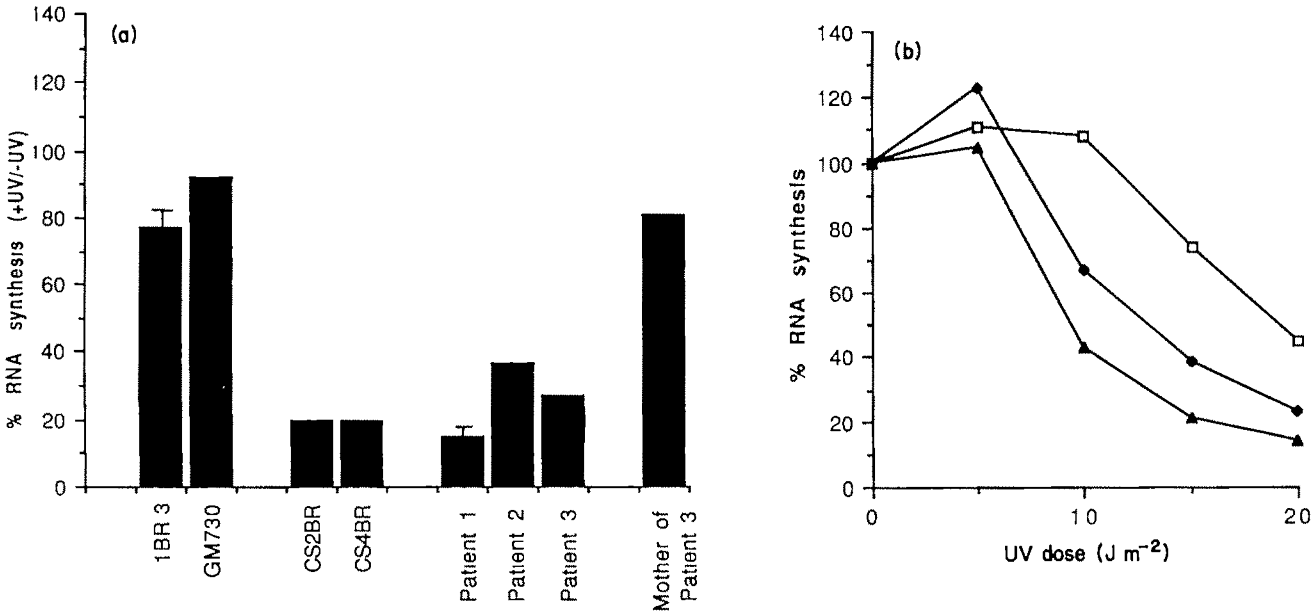

Figure 4. RNA synthesis after UV-irradiation. (a) Non-dividing cells were UV-irradiated (15 J/m^2) and 16 h later were pulse labelled with [^{3}H]-uridine for 1 h. Results are expressed as a percentage of the incorporation in unirradiated cells. 1BR 3 (normal) three experiments; GM730 (normal) two experiments; CS heterozygote (mother of Patient 3) two experiments; CS2BR, (CS) two experiments; CS4BR, (CS) one experiment, Patient 1 (CS) three experiments; Patient 2 (CS) two experiments; Patient 3 (CS) two experiments. The standard error of the mean for 1BR.3 and Patient 1 are indicated by vertical bars. (b) Dose response for post UV-RNA synthesis, —□—, GM730 (normal), —◆—, Patient 2; —▲—, CS2BR.

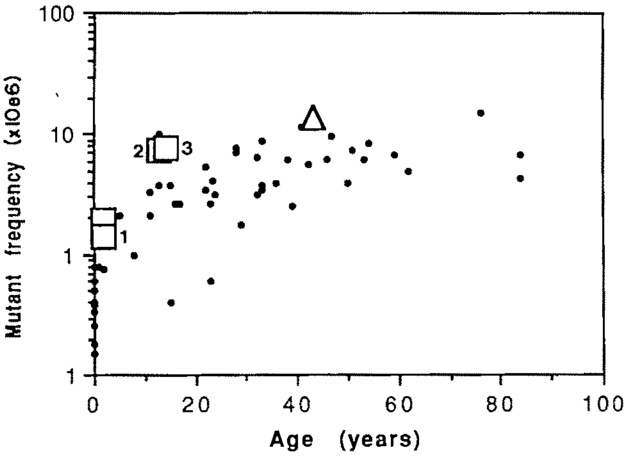

Figure 5. Frequency of 6-thioguanine-resistant mutants in circulating T lymphocytes. ●, normal non-smoking donors; □, Cockayne's syndrome. 1, 2 and 3 refer to Patients 1–3 in Table 4, the unmarked square is patient CSCC; △, Cockayne's syndrome heterozygote (mother of Patient 2).

Table 5. Mutant frequencies for 6-thioguanine resistance in circulating T lymphocytes from Cockayne's syndrome patients and an age-matched series of normal donors

Normal donors		Cockayne's syndrome donors		
Age (years)	MF(×10^{-6})	Designation	Age	MF
1	0·77	Patient 1	2	1·42
2	0·76	CSCC	2	1·86
2	1·86	Patient 2	13	7·28
5	2·08	Patient 3	13	8·05
8	0·97			
11	3·34			
11	2·09			
13	3·80			
13	10·32			
15	0·42			
16	3·78			
16	2·74			
17	2·59			

exposure. It is unclear whether these represent two distinct response patterns or a spectrum of varying sensitivity. Possibly owing to careful sun avoidance, atrophic scarring was lacking in all three of our patients.

Responses to irradiation monochromator testing, previously unreported in Cockayne's syndrome, were abnormal in all three of our cases (Tables 3 and 4). This indicates the potential importance of this investigation as a screening test for CS. However, phototesting does not appear to allow differentiation from xeroderma pigmentosum in which similar abnormalities occur, including a reduced threshold for erythema, delay in

Table 6. A comparison of some clinical and cellular features of Cockayne's syndrome and xeroderma pigmentosum

Characteristic	Cockayne's syndrome	Xeroderma pigmentosum
Acute sun sensitivity	A	A
Delayed erythemal response	A	A
Neurological defect	A*	N→A
Skin cancer	N	A
Adaptive cell-mediated immunity	N	N/A
Natural killer cell function	N	N/A
Fibroblast survival	A	A
Fibroblast induced mutation	A	A
T lymphocyte mutant frequency	A	A
Unscheduled DNA synthesis	N	A†
Daughter-strand repair	N	A‡
Post-UV RNA/DNA synthesis	A	A
Active gene repair	A	A‡

A, abnormal; N, normal.
*Rare exceptions.
†Normal in the XP variant class.
‡May not be defective in some complementation groups.

maximal response beyond 24 h, and abnormal vesicular responses to UVB.[27] The degree of abnormality of phototest responses in CS correlated broadly with *in-vitro* measures of the DNA repair defect and with the severity of acute sun sensitivity but poorly with other clinical features. Otsuka *et al.*[28] similarly found that the degree of UV hypersensitivity of cultured CS cells did not correlate with clinical severity, with the exception of cutaneous photosensitivity. Investigation of the fibroblast response to UVC confirmed the inhibition of post-irradiation RNA synthesis as a characteristic of the cellular response in CS.[9] Indeed, because this had not previously been examined in any of the five cell strains used in this study, our current results may be taken as a cellular confirmation of the diagnosis of CS[10] in these patients. Hypersensitivity to the lethal effects of 254-nm light was confirmed for this syndrome.[5] Further validation of the elevated mutant frequency seen in this series of CS donors will be difficult to achieve because of the rarity of such patients and the tendency of their cells to perform poorly in culture. This latter observation may indeed prove to be a cellular characteristic of the syndrome because we had previously noted[15] difficulties in achieving continued high levels of cloning with firbroblasts derived from CS patients.

Limited observations on two heterozygotes were possible. For one (mother of patient 3), post-UV RNA synthesis and cell survival were normal and for the other (mother of Patient 2), the T lymphocyte mutant frequency was normal. For the present we must regard the heterozygotes as being indistinguishable from normals.

Some of the salient features of CS derived from this and other studies are compared with XP in Table 6. The information here for XP is greatly simplified because many more such patients have been studied and there is an obvious complication caused by having both excision-competent and excision-defective classes.[13]

A major clinical differentiation of CS from XP is the lack of susceptibility to skin cancer. At the cellular level CS can be differentiated by the absence of defects in either excision or daughter-strand repair and, in the two individuals where it has been examined, by normal adaptive cell-mediated immunity and natural killer cell function.[21] The similarly elevated induced mutant frequencies in fibroblasts[15,16] and the increased frequency of mutants amongst circulating T lymphocytes (Fig. 5) suggests that the cells of both CS and XP patients are more susceptible to genetic damage than normals. Thus, the absence of a correlated increase in carcinoma in CS appears to undermine the somatic mutation theory of cancer,[29] which predicts that hypermutability necessarily results in increased carcinogenesis. It was originally proposed by Bridges,[30] however, that an elevated mutation frequency alone was insufficient to understand XP, and he suggested that a second defect, possibly involving immune surveillance might be important. Our demonstration of normal adaptive cell-mediated immunity and natural killer cell function in the hypermutable but non-cancer prone CS suggests that depressed immune surveillance might, in combination with hypermutability, be an essential factor for tumour induction by UV in XP.[31]

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References

1 Cockayne EA. Dwarfism with retinal atrophy and deafness . *Arch Dis Child* 1936; **11**: 1–8.

2 Cockayne EA. Case reports. Dwarfism with retinal atrophy and deafness. *Arch Dis Child* 1946; **21**: 52–54.

3 Neill CA, Dingwall MM. A syndrome resembling progeria. A review of two cases. *Arch Dis Child* 1950; **25**: 213–221.

4 Macdonald WB, Fitch KD, Lewis IC. Cockayne's syndrome. A heredofamilial disorder of growth and development. *Paediatrics* 1960; **25**: 997–1007.

5 Wade MH, Chu EHY. Effects of DNA damaging agents on cultured fibroblasts derived from patients with Cockayne syndrome. *Mutation Res* 1979; **59**: 49–60.

6 Andrews AD, Barrett SF, Yoder FW, Robbins JH. Cockayne's syndrome fibroblasts have increased sensitivity to ultraviolet light but normal rates of unscheduled DNA synthesis. *J Invest Dermatol* 1978; **70**: 237–9.

7 Marshall RR, Arlett CF, Harcourt SA, Broughton BA. Increased sensitivity of cell strains from Cockayne's syndrome to sister-chromatid-exchange induction and cell killing by UV light. *Mutation Res* 1980; **69**: 107–12.

8 Lehmann AR, Kirk-Bell S, Mayne L. Abnormal kinetics of DNA synthesis in ultraviolet light-irradiated cells from patients with Cockayne's syndrome. *Cancer Res* 1979; **39**: 4237–41.

9 Mayne LV, Lehmann AR. Failure of RNA synthesis to recover after UV-irradiation. an early defect in cells from individuals with Cockayne's syndrome and xeroderma pigmentosum. *Cancer Res* 1982; **42**: 1473–8.

10 Lehmann AR, Francis AJ, Giannelli F. Prenatal diagnosis of Cockayne's syndrome. *Lancet* 1985; **i**: 486–8.

11 Venema J, Mullenders LHF, Natarajan AT *et al.* The genetic defect in Cockayne syndrome is associated with a defect in repair of UV-induced DNA damage in transcriptionally active DNA. *Proc Nat Acad Sci USA* 1990; **87**: 4707–11.

12 Cleaver JE. Deficiency in repair replication of DNA in xeroderma pigmentosum. *Nature* 1968; **218**: 652–6.

13 Lehmann AR, Kirk-Bell S, Arlett CF *et al.* Xeroderma pigmentosum cells with normal levels of excision repair have a defect in DNA synthesis after UV-irradiation. *Proc Nat Acad Sci USA* 1975, **72**: 219–23.

14 Maher VM, Ouellette LM, Curren RD, McCormick JJ. Frequency of ultraviolet light-induced mutations is higher in xerodema pigmentosum variant cells than in normal human cells. *Nature* 1976; **261**: 593–5.

15 Arlett CF, Harcourt SA. Variation in response to mutagens amongst normal and repair-defective human cells In: *Induced Mutagenesis Molecular Mechanisms and their Implications for Environmental Protection* (CW Lawrence, ed), New York: Plenum Press, 1982; 249–66.

16 Arlett CF, Cole J. Photosensitive human syndromes and cellular defects in DNA repair. In: *Ozone Depletion: Health and Environmental Consequences* (Russell Jones R, Wigley T eds), Chichester: Wiley and Sons Ltd, Chichester 1989; 147–60.

17 Tates AD, Bernini LF, Natarajan AT *et al.* Detection of somatic mutations in man: HPRT mutations in lymphocytes and hemoglobin mutations in erythrocytes *Mutation Res* 1989; **213**: 73–82.

18 Cole J, Arlett CF, Green MHL *et al.* Measurement of mutant frequency to 6-thioguanine resistance in circulating T-lymphocytes for human population monitoring. In: *New Trends in Genetic Risk Assessment* (Jolles G, ed), New York. Academic Press 1989; 175–203.

19 Kraemer KH, Lee MM, Scotto K. DNA repair protects against cutaneous and internal neoplasia: Evidence from xeroderma pigmentosum. *Carcinogenesis* 1984; **5**: 511–14.

20 Kraemer KH, Lee MM, Scotto J. Xeroderma pigmentosum. Cutaneous, ocular and neurologic abnormalities in 830 published cases. *Arch Dermatol* 1987; **123**: 241–50.

21 Norris PG, Limb GA, Hamblin AS *et al.* Immune function, mutant frequency and cancer risk in the DNA repair defective genodermatoses xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy. *J Invest Dermatol* 1990; **94**: 94–100.

22 Ortaldo JR, Bonnard GD, Herbermann RB. Cytotoxic reactivity of human lymphocytes cultured *in vitro. J Immunology* 1977; **119**: 1351–7.

23 Lehmann AR, Kirk-Bell S, Arlett CF *et al.* Repair of ultraviolet light damage in a variety of human fibroblast cell strains. *Cancer Res* 1977; **37**: 904–10.

24 Henderson L, Cole H, Cole J *et al.* Detection of somatic mutations in man: evaluation of the microtiter cloning assay for T lymphocytes. *Mutagenesis* 1986; **1**: 195–200.

25 Cole J, Green MHL, James SE *et al.* Human population monitoring: a further assessment of factors influencing measurements of thioguanine-resistant mutant frequency in circulating T lymphocytes. *Mutation Res* 1988; **204**: 493–507.

26 Paddison RM, Moosy J, Derbes VJ *et al.* Cockayne's syndrome. A report of five new cases with biochemical, chromosomal, genetic and neuropathologic observations. *Dermatol Trop* 1963; **2**: 195–203.

27 Ramsay CA, Giannelli F. The erythemal action spectrum and doxyribonucleic acid repair synthesis in xeroderma pigmentosum. *Br J Dermatol* 1975; **92**: 49–56.

28 Otsuka F, Tarone RE, Cayeux S, Robbins JH. Use of lympoblastoid cell lines to evalute the hypersensitivity to ultraviolet radiation in Cockayne Syndrome. *J Invest Dermatol* 1984; **82**: 480–484.

29 Burnet FM. Immunological surveillance in neoplasia. *Transplant Rev* 1971; **7**: 3–25.

30 Bridges BA. How important are somatic mutations and immune control in skin cancer? Reflections on xeroderma pigmentosum. *Carcinogenesis* 1981; **2**: 471–2.

31 Lehmann AR, Norris, PG. DNA repair and cancer. speculations based on studies with xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy. *Carcinogenesis* 1989; **10**: 1353–6.

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The treatment of vulval lichen sclerosus with a very potent topical steroid (clobetasol propionate 0·05%) cream

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Summary

The clinical and histological response to 12 weeks of treatment with a very potent topical fluorinated steroid was studied in 15 patients with vulval lichen sclerosus (LS) who were treated with twice daily applications of clobetasol propionate 0·05% cream (Dermovate, Glaxo U.K.). Thirteen patients completed the study and all showed a marked clinical improvement. Histological measurements of skin biopsies taken before and after treatment showed a significant reduction in the characteristic features of LS. One patient developed contact sensitivity to clobetasol propionate. There was no evidence of infection or skin atrophy during the study. Patients completing the study have been followed up for up to 22 months and have been maintained in remission with moderately potent topical steroids which had previously been ineffective.

Vulval lichen sclerosus (LS) is a distressing condition which often fails to respond adequately to therapy. Although many practitioners use potent topical steroids to treat LS there is often concern about the potential harmful effects of these agents on genital skin. This study was carried out to determine the safety and efficacy of the short term but regular use of a very potent topical steroid to control vulval LS.

Methods

Fifteen women with long-standing, biopsy-proven vulval lichen sclerosus gave informed consent to the study. The mean age of the patients was 62 years (range 50–78 years) and the mean duration of disease was 9·3 years (range 2–30 years). Several women had been symptomatic for over 20 years. Most had been treated at some time with a variety of mild or moderately potent topical steroids, anti-candidal preparations and topical oestrogens without effect. Two of the patients had received topical testosterone cream more than 5 years previously and in one patient, local intradermal injections of alcohol had been used more than 10 years before this study.

None of the patients had ever used a very potent topical steroid and none had used a topical steroid of any kind for at least 1 month preceding the study. No patient was receiving systemic steroids or retinoids.

Skin biopsies were obtained from all patients not more than 3 months preceding the study. In most women, a 4-mm punch biopsy was taken from clinically involved vulval skin 1 month before starting treatment. In four women referred from the gynaecology department, incisional biopsies had been performed. After 12 weeks of twice daily application of clobetasol propionate 0·05% cream to the affected areas, a second 4-mm punch biopsy was taken from an adjacent or symmetrically involved area of skin. Both the pre- and post-treatment biopsies were taken from the area with the most severe clinical signs of lichen sclerosus.

Photographs were taken before and after 12 weeks of treatment. Six of the patients made a semi-quantitative measure of pruritus and soreness using 100 mm visual analogue scores (VAS) before treatment and after 6 and 12 weeks of therapy.

All biopsies were examined by one experienced histopathologist without knowledge of the treatment. 100-mm visual analogue scores were used to measure the following features: epidermal atrophy, epidermal basal cell liquefactive degeneration, hyperkeratosis, intensity of inflammatory infiltrate and hyalinization of dermal collagen. The visual analogue scores were analysed using the Wilcoxon matched-pairs signed-rank test. Following the study, the patients have been regularly reviewed for between 2 and 22 months.

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Results

Thirteen patients completed the study. In all these patients the clinical appearance of the vulval skin was markedly improved and in five patients it was no longer possible to make a clinical diagnosis of LS. In the remaining eight women, there was a considerable reduction in erosions, hyperkeratosis, purpura and thickness of plaques. Established labial fusion was not clinically altered by treatment.

Associated with the clinical improvement was a reduction in symptoms of itch and soreness. In the six patients completing visual analogue scores before and after treatment, there was a significant reduction in both symptoms after both 6 and 12 weeks of therapy using the Wilcoxon matched-pairs signed-rank test (Table 1).

The 100-mm visual analogue scores of the histological parameters assessed by a single histopathologist without knowledge of the treatment showed a significant reduction in severity of all the features examined (Table 2). In four of the biopsies taken after 12 weeks of treatment no specific features of LS remained. The histological features of a matched pair of biopsies from one patient taken before (Fig. 1) and after (Fig. 2) illustrate the effects of treatment.

Table 1. Mean 100 mm VAS of pruritus and soreness before and after 6 and 12 weeks of treatment with clobetasol propionate 0·05% cream

	Mean 100-mm VAS			
	Pre-Rx	6 weeks	12 weeks	Significance*
Pruritus	52·3	10·7	2·33	$P=0{\cdot}014$
Soreness	48·3	12·0	5·83	$P=0{\cdot}014$

* Wilcoxon matched-pairs sign-ranked test.

Table 2. Mean VAS of histological features of LS before and after 12 weeks of treatment with clobetasol propionate 0·05%

	Mean 100-mm VAS		
Histological feature	Pre-Rx	Post-Rx	Significance*
Epidermal atrophy	42·71	29·29	$P=0{\cdot}064$
Hyperkeratosis	36·36	20·78	$P=0{\cdot}018$
Epidermal basal cell liquefaction	46·36	16·71	$P=0{\cdot}024$
Intensity of inflammatory infiltrate	41·14	8·07	$P=0{\cdot}0035$
Hyalinization of dermal collagen	62·14	34·43	$P=0{\cdot}022$

* Wilcoxon matched-pairs signed-rank test.

Two patients were withdrawn from the study. One experienced burning discomfort on applying the clobetasol propionate 0·05% cream. Patch tests were performed but no contact allergic reaction to any of the cream constituents could be determined. Another patient did well initially but the treatment was temporarily stopped so that a non-healing erosion could be biopsied to exclude carcinoma. On restarting treatment with clobetasol dipropionate cream, she gave a clear history of itching and eczematous change after its use. Patch testing revealed contact sensitivity to clobetasol propionate cream and ointment and to a fourfold dilution of the clobetasol propionate in petrolatum. There was no reaction to the other constituents of the cream or ointment.

Long-term follow up

At the end of 12 weeks of treatment with clobetasol propionate, patients were changed to a moderately potent topical steroid and were told to apply Dermovate on an 'as required' basis. One patient was lost to follow up after 2 months. The remaining 12 patients have been followed for 6–22 months. All use simple emollients for washing the skin. Three patients still use occasional applications of clobetasol propionate 0·05% (approximately twice a month or less), controlling symptoms at other times with a moderately potent topical steroid cream, clobetasone butyrate 0·05% (Eumovate, Glaxo). Seven patients use clobetasone butyrate as their only treatment either regularly or when necessary. In one patient 1% hydrocortisone controls the residual symptoms.

Two patients now show no clinical evidence of lichen sclerosus. In all the others, the lichen sclerosus appears much less active than before treatment. No patient has active erosions and none has needed a further biopsy.

Discussion

It is not known why lichen sclerosus has such a predilection for genital skin, nor why vulval LS is often so distressingly symptomatic with severe itch and soreness. Vulval LS may be complicated by tissue destruction leading to labial fusion or vaginal stenosis. Squamous carcinoma may develop in association with genital LS although the exact incidence is not clear.[1]

Mild or moderate topical steroid preparations are commonly used to treat vulval LS, but control of symptoms and disease activity is often unsatisfactory. Several studies have reported 2% testosterone propio-

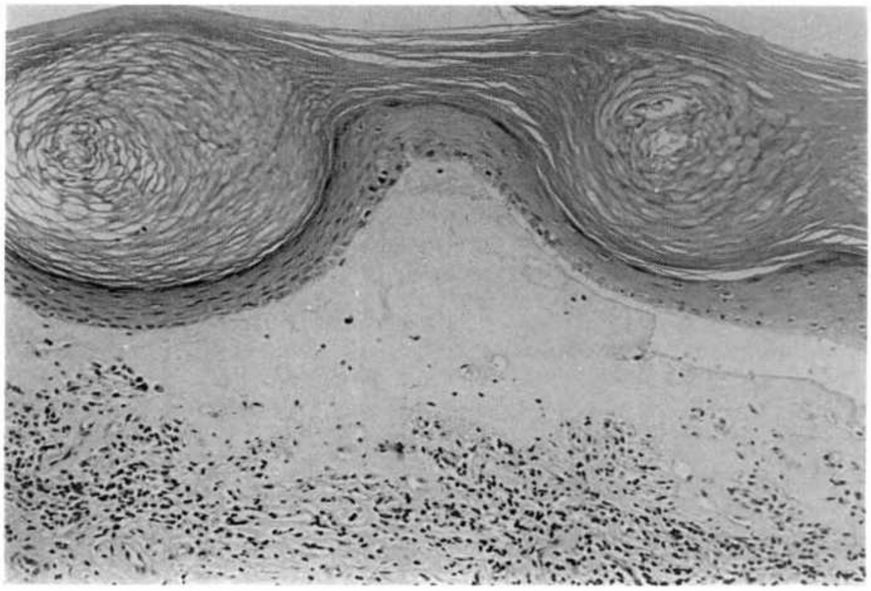

Figure 1. Biopsy from clinically worst affected area of vulval LS before treatment showing hyperkeratosis, epidermal thinning and basal cell degeneration, hyalinization of upper dermal collagen and a mid-dermal lymphocytic inflammatory infiltrate.

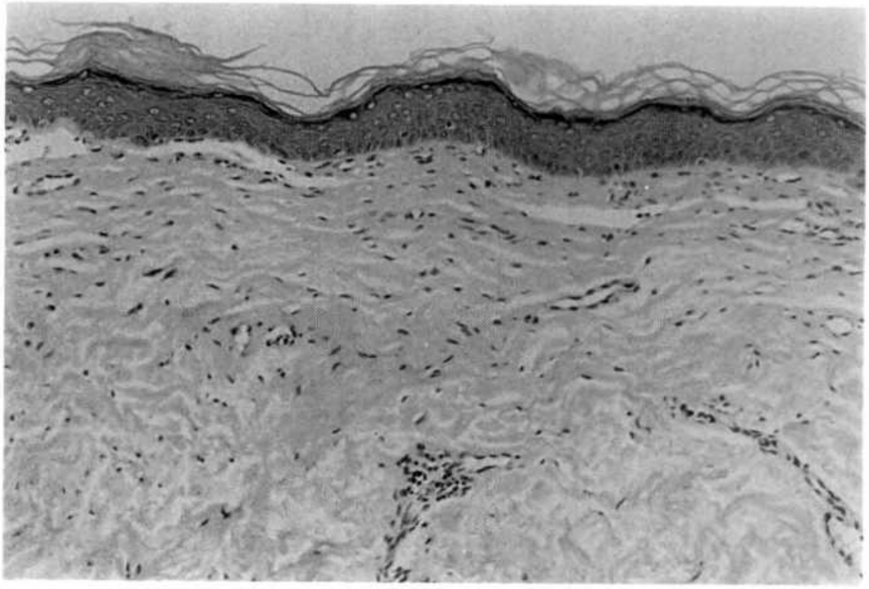

Figure 2. Resolution of features of LS in biopsy from clinically worst affected area of vulval LS after 12 weeks of treatment, in same patient as in Figure 1.

nate cream to be an effective treatment for vulval LS with improvement in histological features.[2] However, this is not readily available, can have virilizing side-effects and is not always effective. In normal vulval skin, the enzyme 5α-reductase metabolizes testosterone to produce amounts of dihydrotestosterone far in excess of that produced in other body sites. Women with vulval LS tend to have lower circulating levels of dihydrotestosterone[3] but whether this is a primary event or secondary to the disease process is unknown. It has been suggested that topical testosterone supplies an excess of substrate inducing local 5α-reductase activity.[4] Systemic therapy of vulval LS with the synthetic retinoid etretinate has produced clinical and histological improvement during treatment but side-effects are common.[5]

It is known that potent topical steroids can relieve symptoms in vulval LS but there has been a reluctance to use them. Concern that they might predispose to local infection was expressed[6] and has often been quoted subsequently. However, there is little evidence to support this, and infection has not been a problem when topical steroids have been used to treat other vulval dermatoses.[7] There has also been concern that the use of fluorinated topical steroids in LS might cause skin thinning in a condition itself characterized by epidermal atrophy.[2] Contact sensitivity induced by clobetasol propionate is well documented[8] and this must be considered when patients report irritation associated with its use.

The long-term follow up of this group of patients demonstrates that once symptoms can be controlled and scratching stops, vulval LS becomes easier to manage and residual symptoms respond to mild or moderate potency steroids. There was no clinical evidence that the use of a very potent steroid predisposed to infection. No clinical or histological evidence of dermal or epidermal atrophy was seen and there was a significant reduction in epidermal atrophy after treatment. Whether better control of the disease will lead to a reduction in the risk of scarring or squamous cell carcinoma remains to be established.

In conclusion, the application of a very potent topical steroid for 12 weeks appears to be safe and effective treatment for vulval LS. The symptomatic relief obtained by the patient is often dramatic and clinical improvement is accompanied by a normalization of histological features.

References

1 Ridley CM. Lichen sclerosus et atrophicus. *Arch Dermatol* 1987; **123**: 457–60.

2 Tremaine RDL, Miller RAW Lichen sclerosus et atrophicus *Int J Dermatol* 1989; **28**: 10–16

3 Friedrich EG, Kalra PS. Serum levels of sex hormones in vulvar lichen sclerosus and the effect of topical testosterone. *N Engl J Med* 1984; **310**: 488–91.

4 Friedrich EG. Vulvar dystrophy. *Clin Obstet Gynaecol* 1985; **28**: 178–87.

5 Mørk N-J, Jensen PJ, Hoel PS. Vulval lichen sclerosus et atrophicus treated with etretinate (Tegison). *Acta Derm Venereol (Stockh)* 1986; **66**: 363–5.

6 Wallace HJ. Lichen sclerosus et atrophicus. *Trans St John's Dermatol Soc* 1971; **57**: 9–30.

7 Bergman A, Karram M, Bhatia NN. Local steroid application for hyperplastic dystrophy of the vulva; clinical and pathological evaluation *J Reprod Med* 1988; **33**: 542–4.

8 Tosti A, Guerra L, Manuzzi P, Lama L. Contact dermatitis from clobetasol propionate. *Contact Dermatitis* 1987; **17**: 256–7.

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Lymphomatoid papulosis followed by large-cell lymphoma: immunophenotypical and genotypical analysis

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Summary The immunophenotype and genotype of atypical cells in skin and lymph node infiltrates were investigated in a patient with lymphomatoid papulosis (LyP) complicated by anaplastic large-cell lymphoma of the lymph nodes. The large atypical cells in both skin and lymph nodes displayed an almost identical immunophenotype, i.e. $CD30^+$ and $CD25^+$. Southern blot analysis for T-cell receptor β-chain gene rearrangement revealed an identical gene configuration in DNA extracted from skin and lymph node. Our results strongly support the hypothesis that clonal populations of T cells arising in cutaneous LyP lesions may undergo malignant transformation, spread into regional lymph nodes, and give rise to secondary malignant lymphomas, such as anaplastic large-cell lymphoma.

Lymphomatoid papulosis (LyP) is a chronic self-healing skin eruption that is clinically benign but histologically mimics a malignant lymphoma.[1] The malignant appearance is due to the presence of a varying proportion of morphologically atypical cells in the dermal lymphoid infiltrate. The clinical course is variable, with progression to disseminated malignant lymphoma in up to 20% of patients.[2–4] A histogenetic relationship among LyP, cutaneous T-cell lymphoma, and Hodgkin's disease has been suggested.[5] Recently it was shown that a high proportion of LyP lesions contain monoclonal expansions of T lymphocytes.[6,7] It has remained unclear whether the malignant infiltrate of subsequently developing lymphoma in the lymph nodes is clonally related to the monoclonal proliferations in the pre- or co-existing LyP lesions. We report a case of LyP with progression to a large-cell lymphoma in a tributary lymph node, where the atypical cells in both skin and lymph node were phenotypically and genotypically identical.

Methods

Case report

A 21-year-old man presented in June 1985 with multiple red-brown papules, nodules, and circumscribed plaques located predominantly on the trunk and the distal forearms. The patient reported spontaneous resolution and reappearance of similar lesions during the previous 2 years. He was otherwise in good health. Several biopsies of small papular lesions were taken for routine histological examination. In August 1988 a larger biopsy was taken from a nodule on the right forearm. At that time no palpable lymph nodes were detected. Two months later, the patient developed lymph node swellings in the right axilla and subsequently in the left groin. Chest X-ray and CT scans of the chest and abdomen as well as abdominal ultrasound showed no additional enlarged lymph nodes. Bone-marrow biopsy revealed no abnormalities. An enlarged axillary lymph node was removed surgically and a histological diagnosis of large-cell lymphoma was made. The patient was treated with five cycles of COP-BLAM (cyclophosphamide, vincristine, adriamycin, bleomycin, procarbazine, prednisone) and IMVP-16 (ifosphamide, methotrexate, etoposide). This produced complete remission of the extracutaneous lymphoma that has lasted up to present time. The cutaneous papular and nodular lesions, however, continue to appear and to resolve spontaneously.

Immunohistology

One-half of each surgically removed specimen from the skin and lymph node lesions was immediately snap frozen in liquid nitrogen and stored at −80°C. The

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remaining portion was routinely processed by formol-fixation and paraffin embedding prior to sectioning. Immunohistological marker analysis on cryostat and paraffin sections was performed as previously described.[8] Briefly, staining with the monoclonal antibodies (MAb) MT-910 (CD2), UCHT-1 (CD3), T3-10 (CD4), KD-23 (CD5), LeuM5 (CD11c), To-15 (CD22), ACT-1 (CD25), Ber-H2 (CD30), Ki-24 (CDw70) as well as the MAb Ki-67, detecting a proliferation-associated nuclear antigen, was performed using the alkaline phosphatase anti-alkaline phosphatase (APAAP) method as previously described.[9,10]

Genotypical analysis

For gene rearrangement analysis, DNA was extracted from frozen skin and lymph node tissue, digested with one of the restriction endonucleases BamHI, BglII, EcoRI, or HindIII (Bethesda Research Laboratories, Karlsruhe, Germany), separated by electrophoresis in 0·7% agarose gels, and transferred to nylon membranes (Hybond N, Amersham, Braunschweig, Germany) as previously described.[11] Probes were labelled with [^{32}P]-dCTP by random primer extension to an activity of 1–3 × 10^9 d.p.m./μg DNA.[12] Hybridization and washing procedures were performed as previously described.[13] The T-cell antigen receptor β-chain (TcR-β) probe, Jurkat-β1,[14] was kindly provided by Dr T.W.Mak, Toronto. A 3·1 kb EcoRI-HindIII immunoglobulin heavy-chain joining region (IgJH) gene probe[15] was obtained by courtesy of Dr G.W.Bornkamm, Freiburg, Germany. The criterion for rearrangement was the appearance of bands in addition to germ-line bands. Completion of digestion was verified by hybridization with a β-globin or bcr gene probe.

Results

Histology

The biopsies from 1985 and 1988 showed a similar morphology with an infiltrate in the upper and lower dermis mainly around blood vessels and the skin appendages. The infiltrate was composed of atypical cells of varying size and morphology. The medium-sized and large atypical cells mostly showed a single prominent rounded or kidney-shaped nucleus with vesicular chromatin structure and single or multiple prominent nucleoli. Occasional multinucleated cells were present. The smaller atypical cells had hyperchromatic cerebriform nuclei. In addition to atypical cells, regularly shaped small lymphoid cells, histiocytes, and few neutrophils were present.

The lymph node specimens showed diffuse and subcortical infiltration with large atypical cells and an admixture of small lymphoid cells and macrophages. The morphology of the atypical cells was identical to that of the large atypical cells in the skin infiltrate.

Immunophenotypical findings

The immunophenotypes of the atypical cell population in the skin lesions of 1988 and in the lymph node are summarized in Table 1. In cryostat sections from the dermal lesions, the large atypical cells were detected by the CD2-, CD25- and CD30-specific antibodies, but were only weakly and inconsistently stained by anti CD3, CD4, and CD5 antibodies. Double immunostaining with the MAbs Ki-67 and Ber-H2 showed that approximately 20% of the larger cells were in the proliferating phase of the cell cycle. Only a few scattered Ki-67$^+$/CD30$^-$ cells were present. The majority of the smaller cells of the infiltrate reacted with CD2, CD3, CD4 and CD5-specific antibodies. The CD11c antigen was detected on a further cell population which was partly dendritic. The immunophenotype of tumour cells in the lymph node was identical to the marker profile of the large atypical cells in the skin (Table 1), showing positivity for CD30, CD25 and CD2 and weak and inconsistent staining with CD3, CD4 and CD5 antibodies. On paraffin sections it could be demonstrated that the CD30 positive large atypical cells

Table 1. Immunophenotype of larger cells in cryostat sections of skin and lymph node infiltrates

Site of biopsy	CD30*	CDw70†	CD2	CD3	CD4	CD5	CD8	CD25	CD11c	Ki-67
Skin	++	++	++	(+)	(+)	(+)	−	++	−	++
Lymph node	+++	+++	(+)	(+)	(+)	(+)	−	+++	−	++

+, occasional cells positive (< 10%); ++, many cells positive (10–60%); +++, large majority of cells positive (60–100%); (+), staining weaker than on reactive T cells

* detected by MAb Ki-1 and Ber-H2.

† detected by MAb Ki-24.

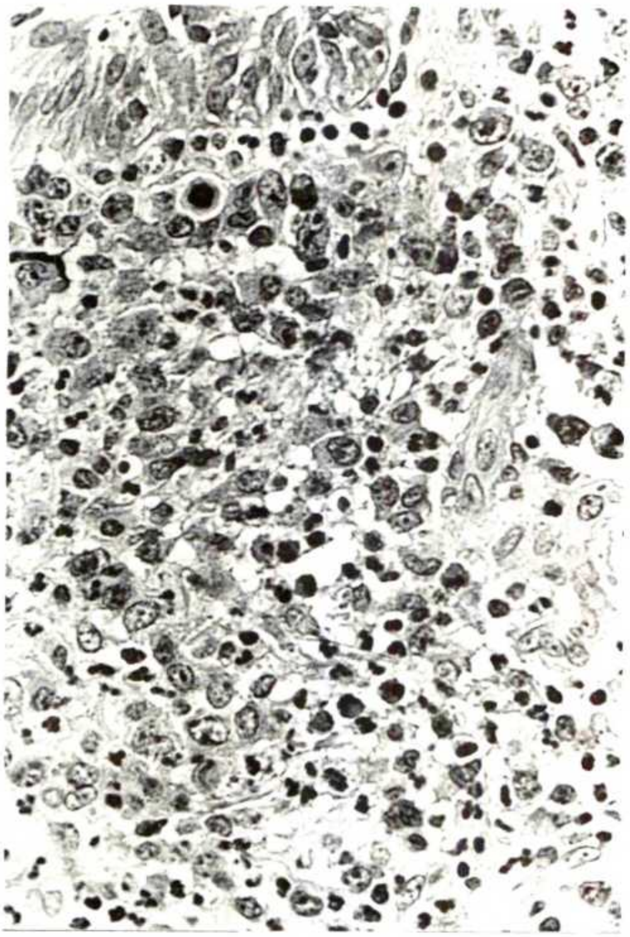

Figure 1. Large atypical cells of cutaneous LyP lesion with prominent nucleoli and smaller atypical cells with cerebriform nuclear contour (haematoxylin and eosin × 320).

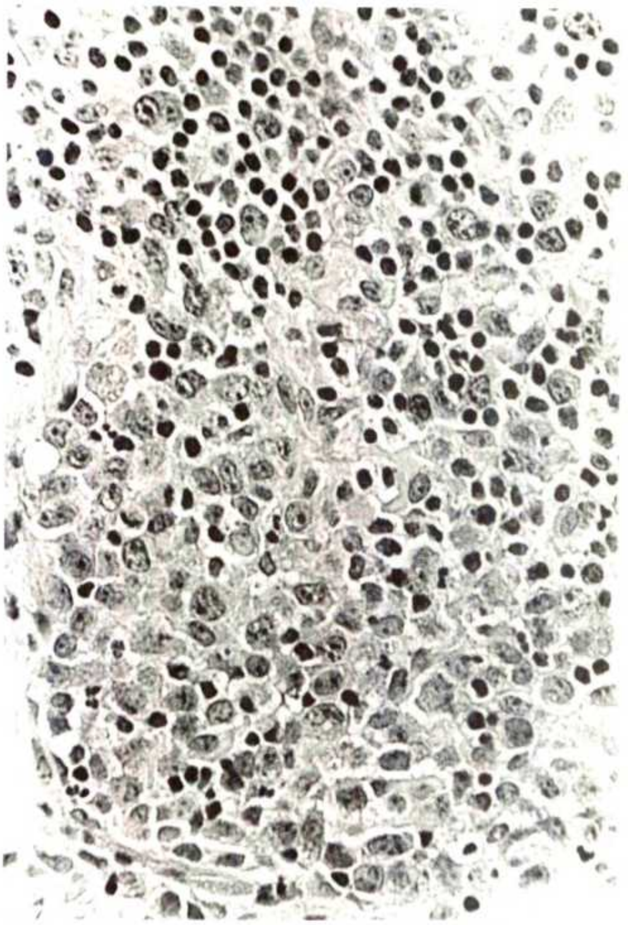

Figure 2. Specific lymph node involvement by large atypical cells (haematoxylin and eosin × 320).

in skin (Fig. 1) and lymph node (Fig. 2) were morphologically closely related.

Gene rearrangement analysis

Southern blot autoradiograms of DNA extracted from both cutaneous and lymph node lesions with the TcRβ and IgJH gene probes showed an identical pattern. A distinct band appearing in addition to the TcRβ gene germline bands indicated a rearranged TcRβ gene configuration, whereas no rearrangements were detected after hybridization with the IgJH gene probe (Fig. 3). TcRβ rearrangements were detectable in both EcoRI or HindIII -digested DNA of both skin and lymph node.

Discussion

Our patient had a 5-year history of recurring and self-healing papular skin lesions which were diagnosed as LyP of the combined types A and B according to the criteria of Willemze *et al.*[16] The patient later developed persistently enlarged lymph nodes which showed infiltration with large atypical cells similar in morphology to those seen in the cutaneous lesions. The immunophenotype of the large atypical cells of the LyP lesion indicated that they were derived from activated T lymphocytes. Double immunostaining for the CD30 antigen and the proliferation-associated nuclear antigen detected by the MAb Ki-67 showed that the CD30-positive cells constituted the largest proliferating cell population in the skin lesions. The neoplastic lymph node infiltrate consisted solely of CD30-positive, large atypical cells with a high growth fraction. This suggests that the atypical cells in the lymph nodes and skin lesion were immunophenotypically closely related and may have been derived from the same malignant T-cell population. The demonstration of identical TcRβ gene configurations is further evidence

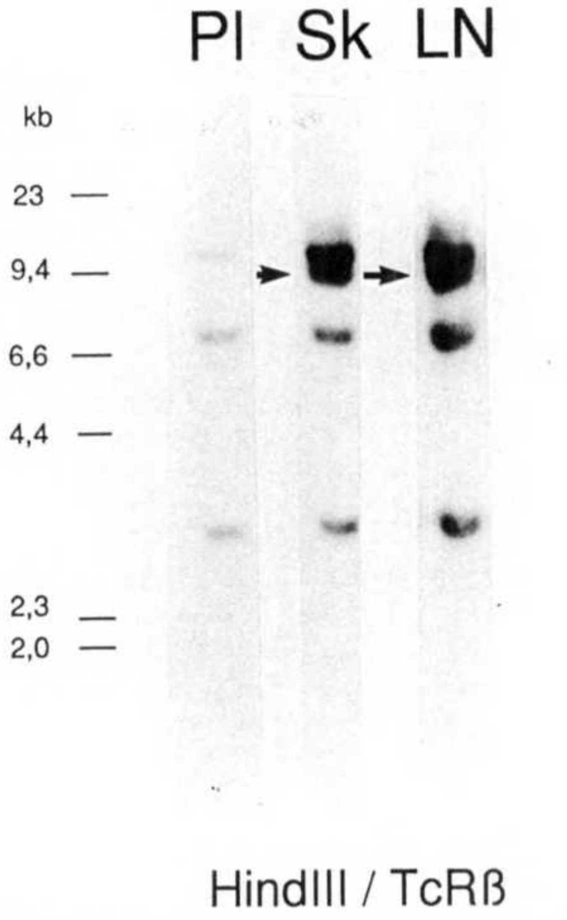

Figure 3. Southern blot analysis demonstrating the same rearranged band in skin (SK) and lymph node (LN) DNA (arrows). PL, placental tissue used as control.

for such a common clonal derivation. We conclude that, in our case, a clonal T-cell population arising in a lesion presenting as LyP has undergone malignant transformation and has disseminated into a tributary lymph node.

Other investigators have found clonal T-lymphoid proliferations in approximately 50% of patients with LyP lesions.[6,7,17] The majority of patients with cutaneous clonal T-cell proliferations displayed a benign course, which shows that demonstration of clonality in cutaneous LyP lesions does not necessarily imply malignancy. In those few patients who developed extracutaneous involvement, genotype analysis was not performed. More recently, the clonal composition of a submandibular mass arising in a patient with a long-standing clinical history of LyP and subsequent development of immunoblastic lymphoma was investigated.[18] In this case, however, the clonal composition of the skin infiltrate could not be determined due to limitations of the cell count.

As both monoclonal and polyclonal T-cell infiltrates are found in LyP lesions it is suggested that LyP may progress into malignant lymphoma by a multi-step process. This includes the development of a monoclonal cell infiltrate predominating in an originally polyclonal cell infiltrate and paralleled by a more aggressive biological behaviour. In some cases this becomes evident in the form of persistent, enlarged cutaneous nodules, which have been described in patients with consecutive malignant lymphoma.[7,19] The appearance of larger papulonodular infiltrates containing monoclonal $CD30^+$ large atypical T cells in LyP may herald the progression of LyP into malignant lymphoma. The detection of such infiltrates by immunophenotyping and genotyping may thus identify those patients with LyP who are at risk of developing large-cell systemic lymphoma.

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References

1 Macauly WL. Lymphomatoid papulosis: a continuing self-healing eruption, clinically benign–histologically malignant. *Arch Dematol* 1968; **97:** 23–30.
2 Tucker WFG, Leonard JN, Smith N *et al.* Lymphomatoid papulosis progressing to immunoblastic lymphoma. *Clin Exp Dermatol* 1984; **9:** 190–5.
3 Messenger AG, Marshall TL, Summerly R. A case of lymphomatoid papulosis and systemic lymphoma. *Br J Dermatol* 1981; **104:** 77–82.
4 Sanchez NP, Pittelkow MR, Muller SA *et al.* The clinicopathologic spectrum of lymphomatoid papulosis: study of 31 cases. *J Am Acad Dermatol* 1983; **8:** 81–94.
5 Kadin ME. Common activated helper-T-cell origin for lymphomatoid papulosis, mycosis fungoides, and some types of Hodgkin's disease. *Lancet* 1985; **ii:** 864–5.
6 Weiss LM, Wood GS, Trela M *et al.* Clonal T cell populations in lymphomatoid papulosis. *N Engl J Med* 1986; **315:** 475–9.
7 Kadin ME, Vonderheid EC, Sako D *et al.* Clonal composition of T cells in lymphomatoid papulosis. *Am J Pathol* 1987; **126:** 13–17.
8 Kaudewitz P, Stein H, Plewig G *et al.* Hodgkin's disease followed by lymphomatoid papulosis: immunophenotypical evidence for a close relationship between lymphomatoid papulosis and Hodgkin's disease. *J Am Acad Dermatol* 1990; **22:** 999–1006.
9 Cordell JL, Falini B, Erber WN *et al.* Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 1984; **32:** 219–29.
10 Stein H, Gatter K, Asbahr H, Mason DY. Use of freeze-dried paraffin-embedded sections for immunohistologic staining with monoclonal antibodies. *Lab Invest* 1985; **52:** 676–83.
11 Herbst H, Tippelmann G, Anagnostopoulos I *et al.* Immunoglobulin

and T-cell receptor gene rearrangement in Hodgkin's disease and Ki-1-positive anaplastic large cell lymphoma: dissociation between phenotype and genotype. *Leuk Res* 1989; 13: 103–16.

12 Feinberg AP, Vogelstein B. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Analyt Biochem* 1983; 132: 6–13.

13 Wahl GM, Meinkoth JL, Kimmel AR. Northern and Southern blots. *Methods Enzymol* 1987, 152: 573–87.

14 Yoshikai Y, Anatoniou D, Clark SP *et al.* Sequence and expression of transcripts of the human T-cell receptor β-chain genes *Nature* 1984; 312: 521–4.

15 Falk MH, Tesch H, Stein H *et al.* Phenotype versus immunoglobulin and T-cell receptor genotype of Hodgkin's disease-derived cell lines: activation of immature lymphoid cells in Hodgkin's disease. *Int J Cancer* 1987; 40: 262–9.

16 Willemze R, Meyer CJLM, van Vloten WA, Scheffer E. The clinical and histological spectrum of lymphomatoid papulosis. *Br J Dermatol* 1982; 107: 131–44.

17 Matsuyoshi N, Horiguchi Y, Tanaka T *et al.* Lymphomatoid papulosis: ultrastructural, immunohistochemical and gene analytic studies. Br J Dermatol 1989; 121: 381–9.

18 Harrington DS, Braddock SW, Blocher KS *et al* Lymphomatoid papulosis and progression to T cell lymphoma: An immunophenotypic and genotypic analysis. *J Am Acad Dermatol* 1989, 21: 951–7.

19 Willemze R. Lymphomatoid papulosis. *Dermatol Clinics* 1985; 3: 735–47.

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Chronic subcorneal pustulosis with vasculitis: a variant of generalized pustular psoriasis in black South Africans

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Summary A rare, but distinctive chronic eruption in six female black South Africans is reported. The original diagnosis of subcorneal pustular dermatosis of Sneddon and Wilkinson in these patients was refuted by the subsequent histological observation of both spongiform pustules and an underlying vasculitis. This may represent a previously undocumented form of generalized pustular psoriasis.

Pustular psoriasis may run an acute or chronic course with or without evidence of previous plaque psoriasis. Skin lesions may be localized, often to the hands and feet, or generalized. Annular forms of generalized pustular psoriasis tend to be subacute or chronic and clinically resemble subcorneal pustular dermatosis of Sneddon and Wilkinson.[1] A subcorneal pustule occurs in both conditions but the spongiform pustule, characteristic of pustular psoriasis, is not found in subcorneal pustular dermatosis.[2]

This is a retrospective study of a rare chronic pustular eruption seen in six black South Africans over the 12-year period 1975–1989. The clinical features and initial histological findings suggested subcorneal pustular dermatosis, but later histological findings included spongiform pustules and evidence of a vasculitis. We have been unable to find vasculitis documented in association with pustular psoriasis (i.e. with spongiform pustules), but it has been reported in patients with acute eruptions precipitated by infection and described as 'bacterids',[3–5] 'pustulosis acuta generalisata'[6] and 'acute acral pustulosis'.[7]

Case reports

All six patients were black South Africans seen as outpatients in the Transvaal between 1975 and 1989. Clinical details are summarized in Table 1. All were female and their ages ranged from 11 to 55 years. Two patients were related, being mother and daughter (Cases 3 and 4), and a third (Case 1) reported that her sister had a similar rash. The duration of the disease varied from 1 to 31 years and the age of onset from 4 to 29 (mean 18) years. Case 4 had never been free of lesions for more than a few weeks at a time and Case 6 had been free of lesions only during periods of treatment with dapsone and prednisone. It is not known whether the rash was continuous or recurrent in the remaining four patients. The lesions were recorded as being itchy in two patients (Cases 2 and 4), not itchy in one (Case 1) and irritating in Case 5. In the remaining two (Cases 3 and 6) the presence or absence of symptoms was not recorded. No patient reported any associated fever, malaise, arthralgia or other systemic upset and all were otherwise healthy with no history of significant previous illnesses.

The eruption affected the trunk and limbs in varying degrees. There was a tendency for involvement of the sides of the neck (Fig. 1) and trunk and the shins. The head, palms and soles were spared in each case. In all patients the skin lesions were at various stages of development. They were diffusely scattered without any grouped, annular or serpiginous arrangement. The primary lesion was a superficial rather flaccid off-white pustule, 2–4 mm in diameter set on a mildly erythematous base. The pustule dried to form a dark crust which separated to reveal a scaling rim around a small white depigmented and slightly atrophic scar. This, together with the surrounding skin, later became hyperpigmented, with some mild residual scaling in areas (Fig. 1). In two patients (Cases 2 and 5) secondary infection was

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Table 1. Clinical and histological findings in patients with chronic subcorneal pustulosis

				Histological features	
Patient	Sex/age (years)	Duration (years)	Distribution of rash	Epidermal	Dermal
1	F/11	1	Trunk, limbs especially shins	Three biopsies:	
				(a) Long flat SCP with basal spongiform pustule and parakeratosis Mild spongiosis	Perivascular oedema + Mild vasculitis
				(b) Flat flaccid SPC Small spongiform pustule Little parakeratosis Mild spongiosis	Papillary oedema + + RBC + + + in papillae Vasculitis
				(c) Round tense intraepidermal SCP Small lateral spongiform pustule No parakeratosis Spongiosis + +	Mild papillary oedema Mild mixed perivascular infiltrate
2	F/25	2	Trunk, mainly lateral; proximal limbs	Tense dome-shaped SCP with PMN and cellular debris Large basal spongiform pustule and parakeratosis	Mild papillary oedema Few papillary RBC Mild mixed perivascular infiltrate
3	F/43	25	Sides of neck, axillae	Long tense SCP with PMN and cellular debris Long basal spongiform pustule and parakeratosis	Marked papillary oedema Papillary RBC + + + Vasculitis
4	F/15	11	Lower legs mainly shins, wrists	Tense round SCP with few PMN Small lateral spongiform pustule Very little parakeratosis	Mild papillary oedema Mild vasculitis
5	F/31	2	Sides of neck, trunk, axillae, groins	Long flaccid SCP Spongiform pustule and parakeratosis in base	Papillary oedema + Perivascular RBC + + Vasculitis + +
6	F/55	31	Sides of neck, trunk, axillae, limbs, shins	Flaccid SCP Long ill-defined basal spongiform pustule No parakeratosis	Papillary oedema Papillary RBC Mild vasculitis

SCP, subcorneal pustule; RBC, red blood cells; PMN, polymorphonuclear leucocytes.

present in some areas resulting in additional frankly purulent pustular and exudative erosions (Fig. 2).

On general examination all the patients looked fit and none showed evidence of systemic disease. It is not certain whether septic foci were specifically sought in each patient, but it was noted that the ears, nose and throat were normal in Cases 2, 5 and 6.

Laboratory investigations

Abnormal findings were as follows. In Case 2 the erythrocyte sedimentation rate was raised to 78 mm/h (Westergren). *Streptococcus pyogenes* and *Staphylococcus aureus* were isolated from lesions which were obviously secondarily infected in Case 2 and *S. aureus* was isolated from an infected lesion in Case 5.

Skin biopsies

Histological examination revealed similar changes in all patients, although it was usually necessary to examine serial sections. The findings in each patient are summarized in Table 1. A constant feature was a subcorneal pustule, either densely packed with polymorphonuclear leucocytes and cellular debris or flaccid and containing relatively few cells. There was a moderate degree of acanthosis and mild spongiosis of the epidermis in most cases. There was evidence of a spongiform pustule in each patient. This was either small and insignificant, situated in the side of a round subcorneal pustule, or large and well developed forming part of the base of a flat blister. A parakeratotic layer was usually seen to form part of the base of the subcorneal pustule (Fig. 3).

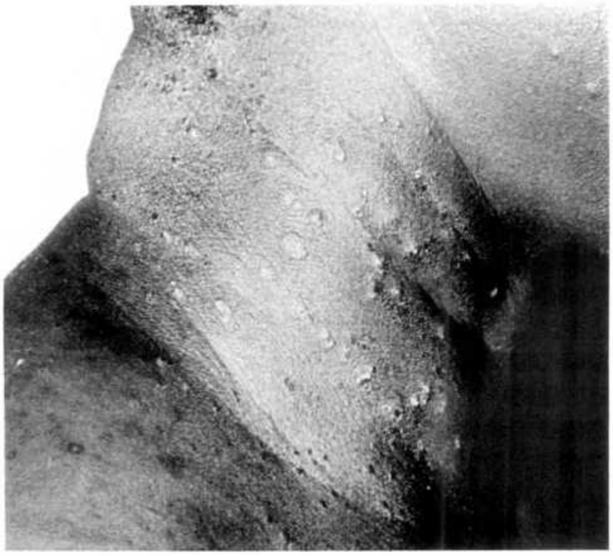

Figure 1. Lesions involving mainly sides of neck in Case 3.

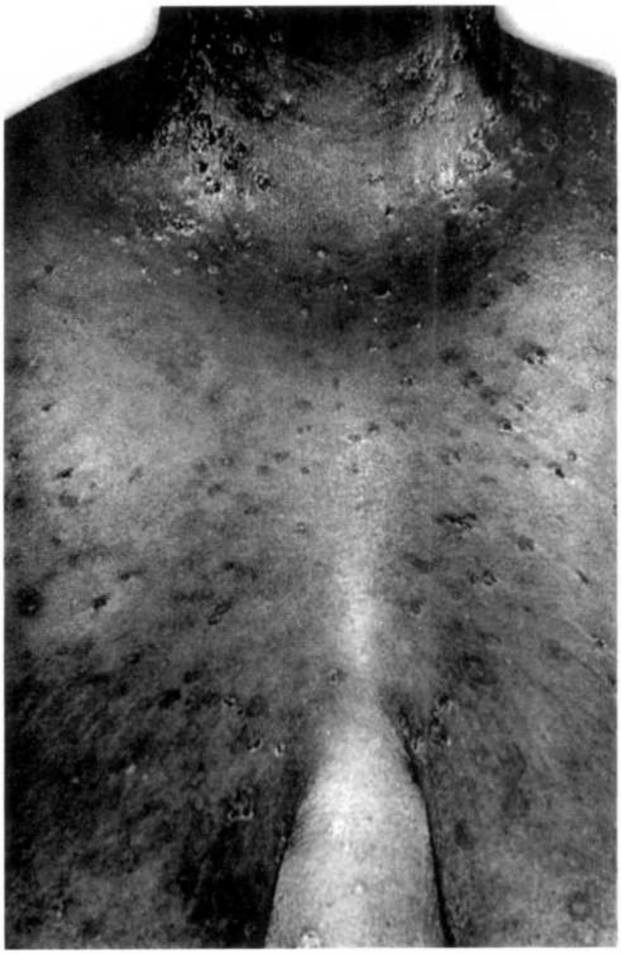

Figure 2. Diffuse involvement of trunk and neck with secondary infection in Case 5.

Dermal changes included papillary oedema which was seen in all patients except Case 4. Extravasation of erythrocytes in the tips of dermal papillae was a feature in some patients and occurred mainly in areas where papillary oedema was intense (Fig. 4). In Case 5 the extravasation was perivascular, situated deeper in the papilla. Some papillae contained polymorphonuclear leucocytes that could be seen migrating through the overlying epidermis towards the subcorneal pustule. Capillaries and larger vessels showed perivascular oedema and a perivascular infiltrate of mononuclear cells and polymorphs showing varying amounts of leukocytoclasis. In Cases 5 and 6 fragments of neutrophils and erythrocytes were seen in the same papillae. Fibrinoid change and disruption of vessel walls were seen in Cases 1, 4, 5 and 6.

Gram stains of pustules in Cases 4 and 6 demonstrated no bacteria.

Immunofluorescence studies on frozen sections in Cases 2, 5 and 6 gave negative or non-specific results. In Case 2 there was a moderately strong, thin linear band of fluorescence at the basement membrane zone under the pustule with C_3 and strong fluorescence of vessels in the mid-dermis with fibrin.

Treatment

In three patients (Cases 1, 2 and 5) the rash responded rapidly to dapsone in a dose of 50–100 mg/day. In Case 6, who was followed up for 2 years, a combination of dapsone 100–150 mg/day and prednisone 5 or 10 mg on alternate days was necessary. The remaining two patients (Cases 3 and 4) showed a rapid temporary improvement on treatment with topical and oral tetracycline, the latter given in a dose of 250 mg twice daily for a few weeks at a time.

Discussion

Our patients were initially diagnosed as having subcorneal pustular dermatosis (Sneddon–Wilkinson) because of the chronic recurrent nature of the eruption and the initial histological finding of subcorneal pustules without other obvious epidermal change. However the finding of a spongiform pustule in one of the later patients in the series prompted us to re-examine serial sections of the original biopsies. In addition to spongiform pustules, we found evidence of capillaritis in the papillary dermis and vasculitis in the mid-dermis. We seemed to be dealing with a specific entity with a characteristic clinical picture in which recurrent wide-

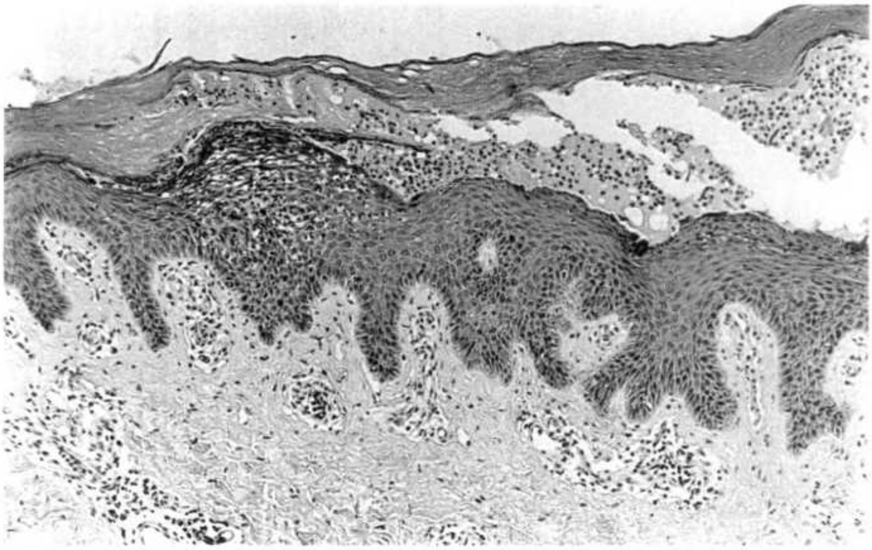

Figure 3. Histology of flat flaccid subcorneal pustule in Case 1 showing spongiform pustule in base with parakeratosis on the right.

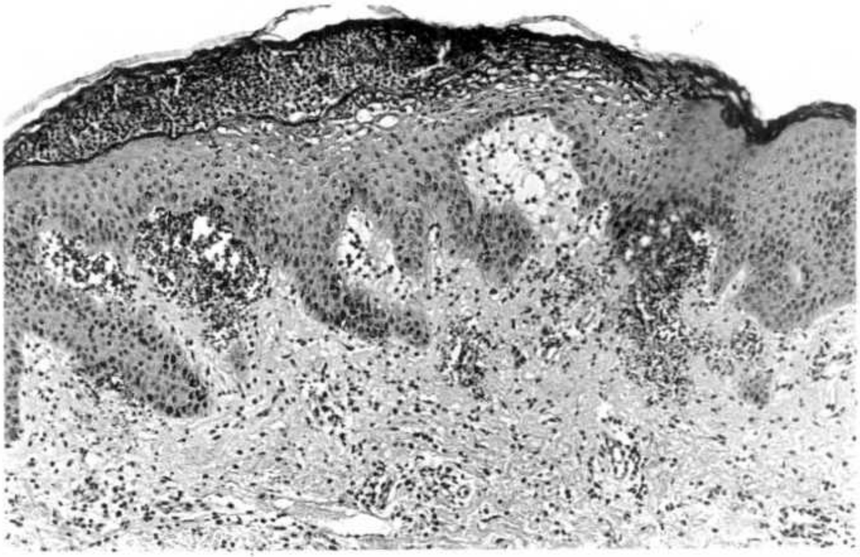

Figure 4. Oedema, erythrocyte extravasation and polymorphs in papillae in Case 3.

spread subcorneal pustules were often maximal in the flexures but were never annular or gyrate and which showed on biopsy a spongiform pustule and underlying vasculitis. Additional features were that all patients were female and that there was a familial occurrence in some. The infection present in some areas in two of the patients was regarded as a secondary phenomenon and not part of the actual disease.

Subcorneal pustular dermatosis was regarded by Sneddon and Wilkinson as a specific entity.[2,8] However, with continued observation many cases have later been diagnosed as pustular psoriasis because they developed spongiform pustules.[9] A spongiform pustule is regarded as the single definitive criterion for distinguishing between subcorneal pustular dermatosis and pustular psoriasis.[2,9] For this reason we consider our patients to have a chronic recurrent form of generalized pustular psoriasis. In our patients both the spongiform and subcorneal pustules varied in size and shape according to the age of the lesion and the level of sectioning. Subcorneal pustules were round and tense with small lateral spongiform pustules in some biopsies while in others they were flat and flaccid with large basal spongiform pustules. In the dermis the presence of marked papillary oedema with erythrocyte extravasation and leucocytoclasis were regarded as evidence of vascular damage.

We have been unable to find vasculitis reported in pustular psoriasis but it has been described in acute pustular eruptions not regarded to be psoriasis. Leucocytoclastic vasculitis with a subcorneal (but not spongiform) pustule was found in an 'acute generalized pustular bacterid' ascribed to a streptococcal throat infection by Tan.[3] The clinical presentation in this case differed from ours in that the pustules were larger, involved mainly the extremities, including palms and soles, and occurred as a single attack of short duration. Miyachi *et al.*[4] described a similar patient with an acute rash in whom histological examination showed spongiform pustules with an underlying leucocytoclastic vasculitis. In addition, there have been three reports of patients who have had upper respiratory tract infections and subsequently developed acute intraepidermal pustular eruptions maximal on their hands and feet.[5-7] These were accompanied by an underlying leucocytoclastic[5,6] or lymphocytic[7] vasculitis and are likely to have been due to circulating immune complexes.

Circulating immune complexes are an expected finding in acute pustular eruptions due to infections but they have also been demonstrated in patients with psoriasis vulgaris where it is uncertain whether they play a primary or secondary role in the pathogenesis of the disease.[10] Depositis of C_3 and immunoglobulins at the dermo-epidermal junction and in vessel walls in patients with pustular and other forms of psoriasis also suggest that immune complexes may be important in the development of lesions.[11] In our patients immune complex deposition is suggested by the observation of a leucocytoclastic vasculitis. The lack of clinical evidence of underlying infection and the chronic recurrent course makes it unlikely that infection played a role. Our immunofluorescence studies serve mainly to confirm the absence of deposits of IgA which have recently been described in other intraepidermal pustular dermatoses.[12]

In our view, these patients represent a variant of pustular psoriasis in which there is a recognizable clinical presentation and in which histological examination reveals evidence of vascular damage in addition to both a subcorneal and spongiform pustule. Since making this observation, we have found this histological combination in biopsy specimens of three patients clinically diagnosed as having pustular psoriasis. Our experience has shown that serial sections and, if necessary, multiple biopsies are needed in order to classify the subcorneal pustular dermatoses properly.

References

1 Baker H, Ryan TJ Generalised pustular psoriasis. A clinical and epidemiological study of 104 cases. *Br J Dermatol* 1968; 80: 771–93.

2 Sneddon IB, Wilkinson DS. Subcorneal pustular dermatosis *Br J Dermatol* 1979; 100: 61–8.

3 Tan RS-H. Acute generalised pustular bacterid. An unusual manifestation of leukocytoclastic vasculitis. *Br J Dermatol* 1974; 91: 209–15.

4 Miyachi Y, Danno K, Yanase K, Imamura S. Acute generalized pustular bacterid and immune complexes. *Acta Derm Venereol (Stockh)* 1980; 60: 66–8.

5 Ishikawa H, Nameki H, Hattori A. Akutes generalisiertes pustulöses Bakterid: Eine Abart des pustulösen Bakterid Andrews. *Hautarzt* 1979; 30: 144–8.

6 Braun-Falco O, Luderschmidt C, Maciejewski W, Scherer R. Pustulosis acuta generalisata. Eine ungewöhnliche Erscheinungsform von leukozytoklastischer Vaskulitis. *Hautarzt* 1978; 29: 371–7.

7 Hoffman TJ, Kettler A, Bruce S Acute acral pustulosis. *Br J Dermatol* 1989; 120: 107–11.

8 Sneddon IB, Wilkinson DS. Subcorneal pustular dermatosis. *Br J Dermatol* 1956; 68: 385–94.

9 Sanchez NP, Perry HO, Muller SA, Winkelmann RK. Subcorneal pustular dermatosis and pustular psoriasis. *Arch Dermatol* 1983; 119: 715–21.

10 Kapp A, Kemper A, Schopf E, Deicher H. Detection of circulating immune complexes in patients with atopic dermatitis and psoriasis. *Acta Derm Venereol (Stockh)* 1986; 66: 121–6.

11 Ullman S, Halberg P, Hentzer B. Deposits of immunoglobulin and complement in psoriatic lesions. *J Cutan Pathol* 1980; 7: 271–5.

12 Wright S, Phillips T, Ryan J, Leigh IM. Intraepidermal neutrophilic IgA dermatosis with colitis. *Br J Dermatol* 1989; 120: 113–19

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A case of candidal umbilical granuloma

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Summary A case is reported of a 14-year-old boy with a mass arising from the umbilicus, which was a large, well-organized candidal granuloma. Cell markers showed normal numbers and distribution of peripheral T and B lymphocytes but there was no reaction to intradermal injection of *Candida*, suggesting a specific T-cell defect such as occurs in chronic mucocutaneous candidiasis.

Case report

A 14-year-old schoolboy presented with a large, protuberant umbilical mass which had arisen from an isolated patch of red, scaly skin over the previous 18 months. There was no history of either acute inflammation or discharge from the lesion. The umbilicus had apparently been entirely normal prior to the development of the mass and had never discharged or leaked fluid. His parents were unrelated and there was no family history suggestive of immune deficiency. There were no significant familial illnesses other than a mild atopic tendency.

During childhood, he was vaccinated against tetanus and poliomyelitis, but not pertussis. Diphtheria immunization was limited to a single dose as it was complicated by marked swelling of the arm. He received BCG vaccination at the age of 13. His general health was good with no significant illness other than childhood eczema.

Examination showed he was of normal height (153 cm) and weight (53 kg). A general examination was normal. There was no evidence of cutaneous disease other than mild facial acne. The scalp, mucous membranes and nails were all normal. Arising from the umbilicus was a soft, brown, pigmented lesion measuring 3 × 5 cm (Fig. 1) with a finely pebbled and mildly scaly surface protruding 3 cm above the surrounding skin.

A wedge biopsy was taken from the mass and the cut surface was pale yellow. On histology there was a granulomatous inflammatory reaction within the dermis. The non-caseating granulomata were composed of epithelioid histiocytes and numerous multinucleated giant cells partially surrounded by a rim of lymphocytes (Fig. 2). The overlying epidermis showed focal parakeratosis but was otherwise unremarkable. Ziehl–Neelsen stain was negative, but PAS demonstrated scanty numbers of fungal hyphae within the granulomata and occasional blastospores in the epidermal keratin. The morphological characteristics of the fungus were those of *Candida* and this was confirmed with positive labelling of fungal hyphae in tissue labelled by indirect immunofluorescence with a rabbit polyclonal antiserum to *Candida*. The hyphae did not label with monoclonal (murine) antibodies to *Aspergillus* or dermatophytes. The mass was then excised. *Candida albicans* was cultured from tissue removed from the centre of the lesion. A 1-month course of intraconazole (100 mg daily) was given as the excision was histologically incomplete.

Initial immunological investigations demonstrated anti-*Candida*-precipitating antibodies present at a titre of 1 : 8 to cell wall mannan and at 1 : 32 to somatic protein/glycoprotein, compatible with active infection and an appropriate serological response. Precipitating antibodies to *Aspergillus fumigatus* were not detected. C-reactive protein was 0·9 mg/dl (normal <0·8), indicating a lack of inflammatory response, compatible with the indolent nature of the lesion. Serum IgG was polyclonally elevated at 24·6 g/l (normal 6·0–13·0) but IgA, IgM, C3 and C4 were all within the normal range. Two months after surgical excision of the lesion, further immunological studies were performed. Serum IgG was 23·0 g/l; total IgE was >1000 kU/l (normal <125), compatible with his atopic background. Specific antibodies were detected at normal titres to tetanus, *Escherichia coli* and *Pneumococcus*; no antibodies were detected against diphtheria, consistent with his incomplete pre-

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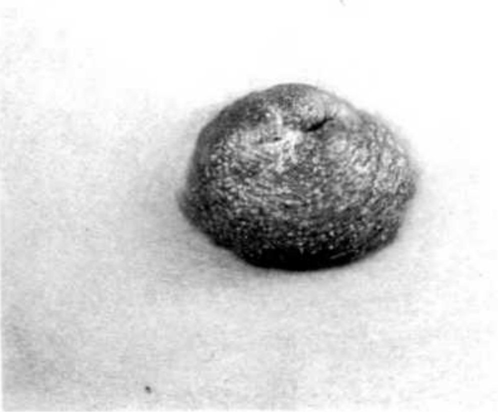

Figure 1. Mass arising from the umbilicus.

vious immunization. Antiviral antibodies were detected against varicella zoster, Epstein–Barr virus and poliovirus (vaccine strain), but not cytomegalovirus, rubella or HIV. Analysis of lymphocyte markers showed normal numbers of $CD4^+$ and $CD8^+$ T cells which were present in normal proportions; B cells were present [$CD20^+$] and almost all were activated as judged by co-expression of CD23. Skin testing for Type IV hypersensitivity was performed using the Merieux Multi-Test CMI: there were weak positive reactions to tetanus and diphtheria antigens, but no response to candidal, streptococcal, tuberculin, *Trichophyton* or *Proteus* antigens. Immunologically, there was evidence for an active humoral response to *Candida*, but no cell-mediated response. Neutrophil function was not studied.

There was a mild iron deficiency anaemia with a haemoglobin of 12·2 g/dl, MCV of 77 fl, MCH of 25·9 pg and a serum ferritin of 49 ng/ml (normal range 30–300 ng/ml). Liver function tests, urea and electrolytes and serum glucose were normal. The serum calcium was 2·47 mmol/l and the serum albumin 44 g/l. Thyroid function tests were normal.

A small bowel enema was performed to exclude the presence of a Meckels diverticulum and patent vitelline duct and was normal. Six months after excision of the umbilical mass, there was no clinical or biochemical abnormality to suggest continuing fungal infection.

Discussion

Chronic *Candida* infection of the skin and mucous membranes is represented by a spectrum of disorders ranging from the banal and easily treated to more aggressive and resistant conditions. Cutaneous candidiasis associated with generalized immunodeficiency is rare, and is usually associated with disseminated candidiasis.[1–3]

Minor nutritional deficiencies of pyridoxine,[4] iron[5] and folate[6] have been shown in some cases to be associated with candidal infection. Some investigators have reported improved immune function following the treatment of iron deficiency,[7] but others have been unable to demonstrate such a response.[5] The nutritional

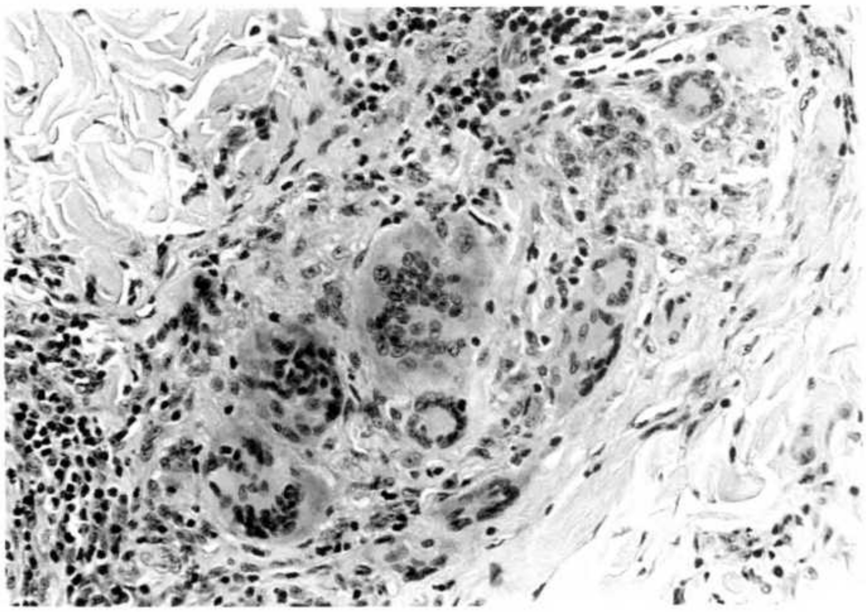

Figure 2. Well-organized granulomata with numerous multinucleated giant cells throughout the lesion.

iron deficiency in our patient may have contributed to the development of the candidal granuloma. Treatment of the iron deficiency occurred simultaneously with surgical removal of the umbilical mass and subsequent therapy with itraconazole. The role of iron deficiency in the pathogenesis of the chronic candidal infection is therefore unresolved in our case.

Immunological investigations in our patient showed a high serum humoral anti-candidal response with negative cutaneous delayed hypersensitivity to injected *Candida* antigen. An immune imbalance was apparent with excess antibody formation and defective cell-mediated immunity. Three mechanisms have been postulated to explain this phenomenon:[8] delay in antibody response to the organism may allow rapid multiplication of *C. albicans* with subsequent production of excess anti-candidal antibody; high levels of *Candida* antibody may also result from previous infections; a third possibility is an excessive humoral response following the initial candidal infection. All three mechanisms result in high levels of *Candida* antibody which may impair cell-mediated immunity to *Candida*.

Eight cases of resistant oral candidiasis were reported by MacKie *et al.* in 1978.[8] Four of these patients had no significant abnormality in immune function and responded to antifungal therapy with nutritional supplements. The remaining four patients had deficiencies in specific *Candida*-related immune function, as seen in our case, with no evidence of a more generalized immunodeficiency. *Candida*-killing and nitro-blue-tetrazolium tests were not performed on our patient as immunological assessment was directed at humoral and cell-mediated aspects of lymphocyte function. The eight patients described above all had *Candida*-killing and nitro-blue-tetrazolium tests which were normal, suggesting normal neutrophil function.

A specific defect in the immune system may rarely result in disseminated candidiasis in otherwise immunocompetent patients. Staples *et al.*[9] reported the case of a previously healthy 15-year-old girl who developed a nearly fatal *Candida albicans* septicaemia with peritonitis. Immunological investigation revealed normal humoral and cell-mediated *Candida* responses and normal phagocytosis of *Candida albicans*, but candidacidal assays using the patient's peripheral blood leucocytes gave significantly impaired values. It was concluded that a unique leucocytic defect had been identified.

A comparison of our case with cases of chronic mucocutaneous candidiasis (CMC) shows clear differences in clinical, histological and immunological features. The histology of 'granulomata' in CMC characteristically shows massive hyperkeratosis with little or no formation of granuloma, and is therefore something of a misnomer.[10] The well-organized fungal granuloma seen in our case is histologically far removed from the typical appearances seen in CMC. Such patients also have much higher levels of anti-*Candida* IgG than was seen in our case and commonly have additional defects in immunological function.[11] Clinically there were striking differences, between our case and CMC. Chronic mucocutaneous candidiasis includes a spectrum of disease entities ranging from a severe combined immune-deficiency commonly fatal in childhood, to the mildest form in which late-onset candidiasis may be limited to paronychia or buccal mucosal involvement.[12] An associated endocrinopathy may occur in CMC and was not identified in our patient.

Granulomatus lesions have been reported in children in the napkin area[13] and gluteal region.[14] The reddish-brown tumours described in these reports resemble our case apart from being multiple in number and smaller in size. In two cases[13] *C. albicans* was isolated from the surface of the lesions, but histologically no fungal elements were seen and foreign body giant cells were absent. The histological features of the gluteal granulomata[14] were dissimilar to our case, with fibrous proliferation and fibrosarcomatous features with spindle cells. Neither of these two reports[13,14] implicated *C. albicans* in the pathogenesis of the granulomatous lesions.

The differential diagnosis of umbilical granulomata includes a variety of conditions, including talc granuloma. Eighteen cases of talc granuloma of the umbilicus were reviewed in 1970,[15] of whom 13 represented pyogenic granulomata, and five umbilical polyps. The clinical diagnosis made at presentation of our patient was that of a talc granuloma but no histological evidence of talc could be found.

The absence of a history of discharging urine and a cutaneous opening excluded the diagnosis of a patent urachus in our patient. A patent vitelline duct may cause an inflammed umbilicus but more commonly presents as an umbilical polyp in later life.[16] A small bowel enema was performed to exclude this rare possibility in our case.

Acknowledgments

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References

1 Jarowski CI, Fialk MA, Murray HW *et al.* Fever, rash and muscle tenderness: a distinctive clinical presentation of disseminated candiasis. *Arch Intern Med* 1978; **138:** 544–6.

2 File TM Jr, Marina OA, Flowers FP. Necrotic skin lesion associated with disseminated candidiasis. *Arch Dermatol* 1979; **115:** 214–15.

3 Grossman ME, Silvers DN, Walther RR. Cutaneous manifestations of disseminated candidiasis. *J Am Acad Dermatol* 1980; **2:** 111–16.

4 Axelrod AE, Trakatellis AC. Relationship of pyridoxine to immunological phenomena. *Vitamins and Hormones* 1964; **22:** 591–607.

5 Higgs JM. Chronic mucocutaneous candidiasis: iron deficiency and the effects of iron therapy. *Proc Royal Soc Med* 1973; **66:** 802–4.

6 Jenkins WMM, MacFarlane TW, Ferguson MM, Mason DK. Nutritional deficiency in oral candidosis. *Int J Oral Surg* 1977; **6:** 204–10.

7 Dagg JH, Jackson JM, Curry B, Goldberg A. Cytochrome oxidase in latent iron deficiency (sideropenia). *Br J Haematol* 1966; **12:** 331–3.

8 MacKie RM, Parratt D, Jenkins WMM. The relationship between immunological parameters and response to therapy in resistant oral candidosis. *Br J Dermatol* 1978; **98:** 343–8.

9 Staples PJ, Boujak J, Douglas RG Jr, Leddy JP. Disseminated candidiasis in a previously healthy girl: implication of a leukocyte candidacidal defect. *Clin Immunol Immunopath* 1977; **7:** 157–67.

10 Hauser FV, Rothman S. Monilial granuloma: report of a case and review of the literature. *Arch Dermatol Syphil* 1950; **61:** 297–310.

11 Wells RS, Higgs JM, MacDonald A *et al.* Familial chronic mucocutaneous candidiasis. *J Med Genetics* 1972; **9:** 302–10.

12 Stavaren AM, Stiehm ER. Chronic mucocutaneous candidiasis: clinical, immunologic and therapeutic considerations. In: *Paediatrics Update* (Moss A J, ed). New York: Elsewier, 1986; 93–110.

13 Uyeda K, Nakayasu K, Takaishi Y, Sotomatsu S. Kaposi sarcoma-like granuloma on diaper dermatitis. *Arch Dermatol* 1973; **107:** 605–7.

14. Tappeiner J, Pfleger L. Granuloma glutaele infantum. *Hautarzt* 1971; **22:** 383–8.

15 McCallum DI, Hall GFM. Umbilical granulomata — with particular references to talc granuloma. *Br J Dermatol* 1970; **83:** 151–6.

16 Hejaki N. Umbilical polyp: a report of 2 cases. *Dermatologica* 1975; **150:** 111–15.

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Multiple keratoacanthomas: a case report with evidence of regression with thymic hormone

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Summary A case of multiple keratoacanthomas (KAs) arising on sun-damaged skin in a 63-year-old male is reported. The patient showed a moderate elevation of the T-helper/T-suppressor ratio. Therapy with thymic hormone appeared to improve the condition.

Keratoacanthomas (KAs) are benign, self-healing epithelial tumours of the skin which usually arise as a single lesion in sun-exposed areas. When multiple, they may be familial or sporadic,[1–3] and associated with immunological abnormalities[4] and as a consequence of intense sun exposure.[5] We report a case of multiple KAs arising in a patient with a long history of sun exposure and slight abnormalities of the lymphocyte subset.

Case report

A 63-year-old fair-skinned man had been a butcher since he was 15 years old, but at the age of 33 started working as a farmer in his spare time. At the age of 43 the first KA appeared on his right cheek and since then new lesions have continued to appear at an average rate of one per year. When he was 48, the patient started to work full-time as a farmer and KAs then appeared at an average rate of four per year. The patient would usually attend hospital 4–8 weeks after the appearance of the lesions which were then excised. Later on the KAs were left *in situ* and underwent spontaneous involution after 10 months. Currently, 20 years after the appearance of the first lesion the patient has developed 43 KAs in total. Thirty-four of these were excised, seven spontaneously regressed and two are still present. Most of the lesions were located on the face (26), seven were on the forearms and nine on the legs. Both sides of the body were equally affected. One KA was present on the epidermal side of the anorectal junction. The patient was otherwise in good health. Yearly chest X-rays, gastrointestinal endoscopy and laryngeal examination were negative. Routine serum and blood tests were normal.

In June 1989 the peripheral lymphocyte subsets were studied using a cytofluorimeter FACScan (Becton-Dickinson) (Table 1). As the number of T-suppressor lymphocytes was low, the patient was treated with thymic hormone (70 mg/day for 15 days, and then 70 mg twice weekly for 3 months). In addition a sunscreen was prescribed. Three KAs which had appeared before treatment rapidly healed. Repeat typing of lymphocytes at the end of the treatment revealed almost normal subset levels (Table 1). Four months after suspension of therapy the patient presented with two new lesions on the right forearm and on the upper lip. A second cycle of therapy was started and the lesions disappeared within 8 weeks. The lymphocytes were typed 2 weeks after the beginning of this new cycle of therapy (Table 1). No lesions were evident 1 month after the disappearance of the last lesion.

The macroscopic features varied according to the age of the lesions. Initially they appeared as small reddish nodules that enlarged within 6–8 weeks (measuring 0·5–2·5 cm in diameter), while a central cavity

Table 1. Percentages of lymphocyte subsets

	Percentage of total lymphocytes			
Lymphocyte subset	Before therapy	After therapy	During therapy (second cycle)	Normal values
T helper	63·6	52	58	29–55
T suppressor	17·3	24	22	19–47
Th/Ts	3·7	2·16	2·63	0·8–1·7
Mature T	62·4	66	65	63–79
Mature B	8·6	12	11	10–18

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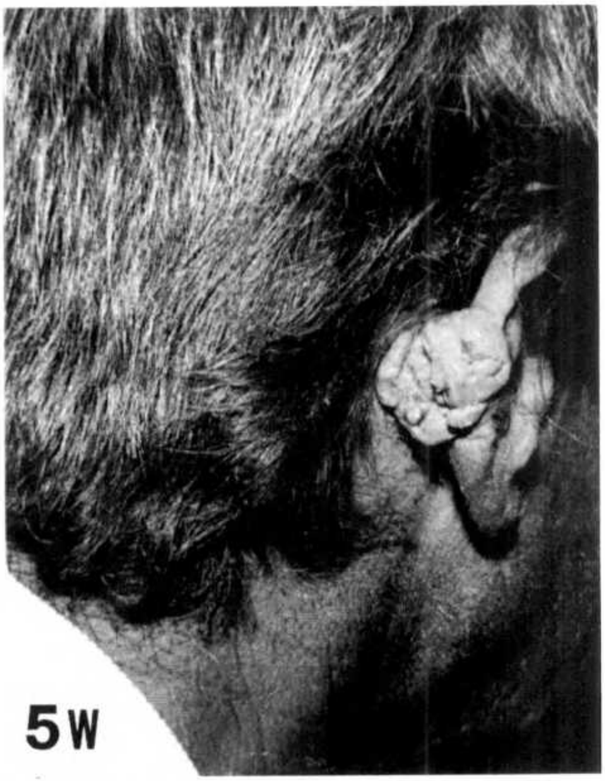

Figure 1. A 5-week-old KA appears as a nodule with a central horn-filled crater.

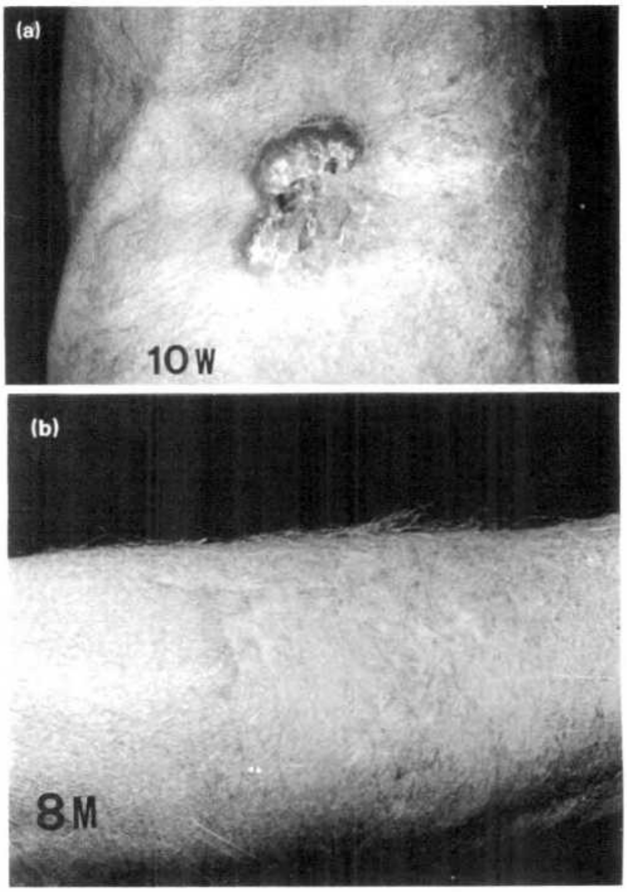

Figure 2. (a) Partially healed 10-week-old KA. (b) KA 8 months after first appearance, appearing as whitish flat scar.

appeared (Fig. 1). Lesions which had been left *in situ* for 9–16 weeks were larger, reaching 8·5 cm in diameter, and had a central horn-filled crater. Lesions present for more than 16 weeks appeared flat, white and were covered with keratin. After 6–10 months KAs resolved into whitish, flat scars (Fig. 2). This pattern was reflected histologically. Nodules that were immediately excised showed a cental keratin-filled invagination of the epidermis, surrounded by strands of epithelial cells (Fig. 3). In well-developed lesions the central horn-filled crater was enlarged. Peripheral groups of epithelial cells branched from the central crater and invaded the underlying dermis, without extending below the plane of the sweat glands. In two lesions epithelial cells infiltrated perineural spaces. Elastic fibres were sometimes observed within the epithelium. Epithelial cells surrounding the crater and composing the strands did not show atypical features or mitoses (Fig. 4). A prominent inflammatory infiltrate was present around the well-developed lesions. In older lesions the central crater was flattened. The skin adjacent to the KAs showed features of actinic keratosis of the hypertrophic type.

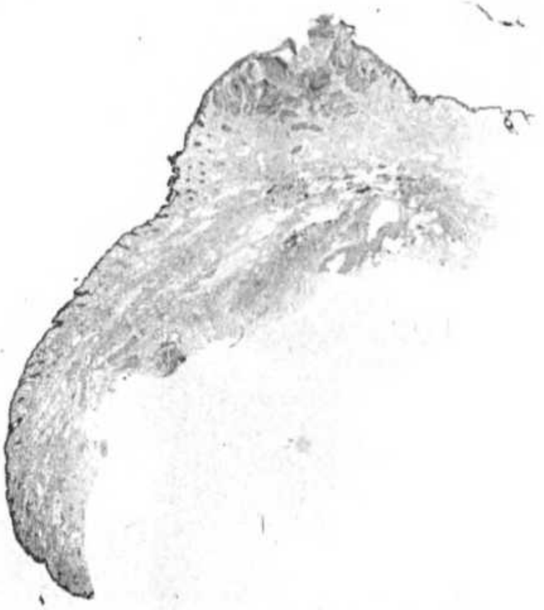

Figure 3. Well developed KA showing typical central crater, surrounded by branches of epithelial cells (haematoxylin and eosin, ×2·5).

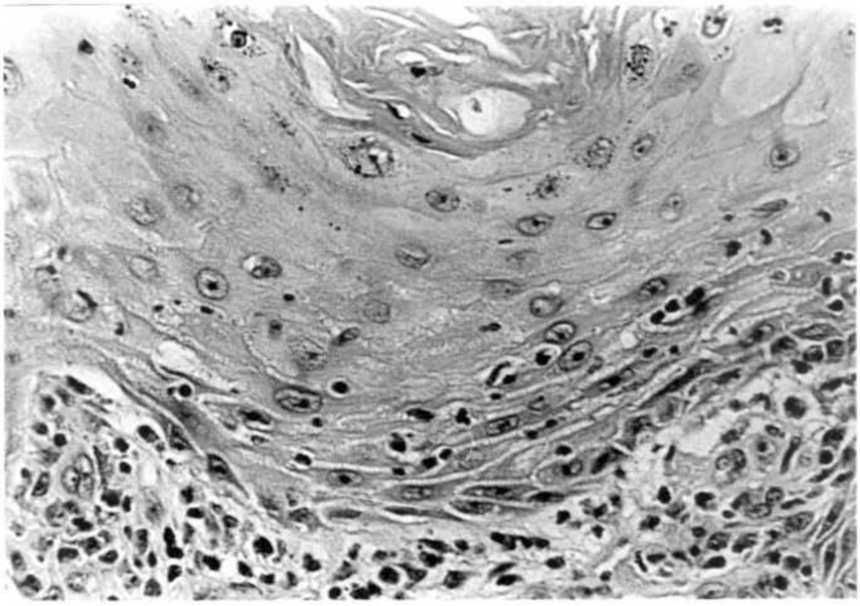

Figure 4. Higher power view of a well developed KA, epithelial cells do not show atypical features or mitotic figures (haematoxylin and eosin, × 350).

Discussion

To date our patient has developed 43 KAs, mostly on the sun-exposed areas of the skin. The size and high number of the lesions in cases of multiple KAs often lead to the suspicion of malignancy. However, the histological features of the lesions in our patient were typical of KAs. In two lesions epithelial cells infiltrated the perineural spaces which is a well-documented feature in KAs.[6] The 20-year history of multiple excisions, without signs of recurrence, and the spontaneous regression of seven lesions also indicate a benign process.

Several forms of multiple KAs have been reported in the literature. The most frequently reported form is the eruptive type of KA,[7] characterized by hundreds of minute, synchronous KAs with generalized involvement of the skin and mucous membranes and frequent association with internal diseases, such as hypertension and nephrosclerosis.[8] Our patient had less numerous KAs, which did not involve the mucous membranes and did not arise synchronously. The Ferguson Smith syndrome[2] is an autosomal dominant condition affecting young patients, characterized by multiple KAs which may occur in crops. Muir–Torre syndrome[1,3] is another familial form of multiple KAs associated with sebaceous tumours and primary carcinomas of the gastrointestinal tract, larynx and lung. The lack of family history and of internal malignancies in our patient excluded these two syndromes.

In 1939, Poth[5] reported a case of multiple KAs arising in a fair-skinned man with a history of sun exposure. The KAs disappeared when the patient avoided sun exposure. Since then eight similar cases have been reported in the literature (Table 2). In six of these cases a causative factor, such as PUVA, sun and heat, was present but in three cases no such factors were traced.[4,11,12] In our case the appearance of KAs was related to sun exposure and only one of the 43 KAs was in a non-exposed site, near the anogenital region. Multiple KAs have been reported in immunodepressed patients[14] but there was no evidence of either iatrogenic or spontaneous immunosuppression in our patient. The only anomaly of the immune system that we found was an increased T-helper/T-suppressor ratio. A study of mice exposed to UV rays found local acute effects, and distant effects, mediated by an increased number of T-suppressor lymphocytes.[15] These results were confirmed in humans[16] when an increased number of T-suppressor lymphocytes was found in sun-exposed patients with multiple skin cancers. A study of peripheral lymphocyte subsets in a patient with eruptive KAs found, unlike in the present case, that the T-helper/T-suppressor ratio was normal but mitogen-induced lymphocyte proliferation was low.[17] Clinical improvement following treatment with etretinate was accompanied by a normal response of mitogen-induced lymphocyte proliferation.

We cannot state, at present, whether the increased T-helper/T-suppressor ratio in our patient was a consequence of the chronic sun exposure or part of the causation for the multiple KAs. Variations of the T-

Table 2. Cases of multiple keratoacanthomas

Authors	Sex	Age	Exposure	No. of lesions	Sites	Dimensions (cm)	Follow up
Poth[5]	M	45	Sun	4	Hands	2	FU/NT
Fliegelman and Loveman[9]	M	75	Heat	>4	Hands	2	DOC
Ephraim and Kaufman[10]	F	65	Sun	20	Arms, forearms	3	AWD
Bellone[11]	F	29	NS	30	Face, back	1·5	AWD
Reid and Cheesbrough[4]	M	62	NS	19	Eyelids, back, legs, feet, hands, penis	2·3	AWD
Colombo *et al.*[12]	M	71	NS	5	Face, forearms	NS	AWD
Sina and Adrian[13]	M	43	PUVA	14	Face, trunk, extremities	2	AWD
	M	55	PUVA	'Multiple'	Legs	NS	No FU
Present case	M	63	Sun	43	Face, forearms, legs, anus	2·5	AWD

FU, follow up; NT, no therapy; AWD, alive with disease, DOC, died of other causes; NS, not stated.

helper/T-suppressor ratio may not be specific as they occur in several pathological conditions.[18] The normalization of the T-helper/T-suppressor ratio after treatment with thymic hormone corresponded to a period of freedom from new KAs and the rapid regression of three KAs; however more cases will need to be studied.

Acknowledgments

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References

1 Muir EG, Bell AJY, Barlow KA. Multiple primary carcinomata of the colon, duodenum and larynx associated with keratoacanthomata of the face. *Br J Surg* 1967; 54: 191–195.

2 Smith F A case of multiple squamous-celled carcinomata of the skin in a young man, with spontaneous healing. *Br J Dermatol Syphil* 1934; 46: 267–72.

3 Torre D. Multiple sebaceous tumors. *Arch Dermatol* 1968; 98: 549–51.

4 Reid BJ, Cheesbrough MJ. Multiple keratoacanthomata. A unique case and review of the current classification. *Acta Derm Venereol (Stockh)* 1978; 58: 169–73.

5 Poth D. Tumor-like keratoses. Report of a case. *Arch Dermatol* 1939; 39: 228–36.

6 Ianecka IP, Wolff M, Crikelair GF *et al.* Aggressive histological features of keratoacanthoma. *J Cutaneous Pathol* 1978; 4: 342–8.

7 Grybowski M. A case of peculiar generalized epithelial tumours of the skin. *Br J Dermatol* 1950; 62: 10–13.

8 Winkelman RK, Brown J. Generalised eruptive keratoacanthoma. Report of cases. *Arch Dermatol* 1968; 97: 615–23.

9 Fliegelman MT, Loveman AB. Tumor-like keratoses. *Arch Dermatol Syph* 1952; 66: 353–7.

10 Ephraim A, Kaufman JJ. Multiple keratoacanthoma. Report of an extensive case and its response to therapy. *AMA Arch Derm* 1958; 77: 191–4.

11 Bellone AG. Cheratoacantoma multiplo. *Giorn It Derm Ven* 1960; 101: 256–76.

12 Colombo E, Mossini A, Griffanti P *et al.* Su di un caso di chertoacantoma multiplo localizzato. *Giorn It Derm Ven* 1982; 117: 173–4.

13 Sina B, Adrian RM. Multiple keratoacanthomas possibly induced by psoralens and ultraviolet A photochemotherapy. *J Am Acad Dermatol* 1983, 9: 686–8.

14 Rook A, Wimster I. Keratoacanthoma, a thirty year retrospect. *Br J Dermatol* 1979; 100: 41–7.

15 Kripke ML, Morison WL. Modulation of immune function by UV radiation. *J Invest Dermatol* 1985; 85: 62s–66s.

16 Frenz G, da Cunha Bang F, Munch-Petersen B, Wantzin GL. Increased number of circulating suppressor T-lymphocytes in sun-induced multiple skin cancers. *Cancer* 1988; 61: 294–7.

17 Blitstein-Willinger E, Haas N, Nurnberger F, Stuttgen G. Immunological findings during treatment of multiple keratoacanthoma with etetrinate. *Br J Dermatol* 1986; 114: 109–16.

18 *Harrison's Principles of Internal Medicine.* (Wilson JD, Braunwald E, Isselbacher KJ, Petersdorf RG, Martin JB, Fauci AS, Root RK, eds), 12th edn. New York: McGraw-Hill Inc, Health Professions Division, 1990; 86.

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Oral hairy leukoplakia in a HIV-negative renal graft recipient

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Summary Oral hairy leukoplakia (OHL) is seen almost exclusively in patients infected with HIV. A case is reported of OHL occurring in a patient who was seronegative for HIV and who had a renal graft. This occurred following an increase in his treatment with immunosuppressive drugs.

Oral hairy leukoplakia (OHL) was first described in 1984 in male homosexuals with AIDS[1] and subsequently in other groups of HIV-infected patients. The condition is due to an opportunistic infection with the Epstein–Barr virus (EBV) in the keratinocytes of the oral mucosa. It has generally been considered as a hallmark for HIV infection. We have recently observed a typical case of OHL that developed in a HIV-negative patient who had a renal graft and who was on immunosuppressive therapy.

Case report

A 40-year-old Caucasian male with polycystic renal and hepatic disease and who developed chronic renal failure had in July 1989 a cadaveric renal transplant. He was commenced on treatment with 15 mg prednisolone, 100 mg azathioprine and 200 mg cyclosporin daily. In late December, because of graft rejection, the dose of prednisolone was increased to 65 mg daily. In mid-January 1990 he developed white irregular and raised plaques on the lateral borders of the tongue (Fig. 1a). A diagnosis of candidosis was made by his family doctor but treatment with fluconazole was ineffective. He was later seen by one of us (S.E.) and the correct diagnosis of oral hairy leukoplakia was made. He was otherwise in good health and had no other infection. Serological tests for HIV-1 and HIV-2 before and after the appearance of the OHL lesions were all negative.

In late February 1990 the dose of steroids was reduced and the lesions of OHL cleared completely (Fig. 1b), and there has been no recurrence.

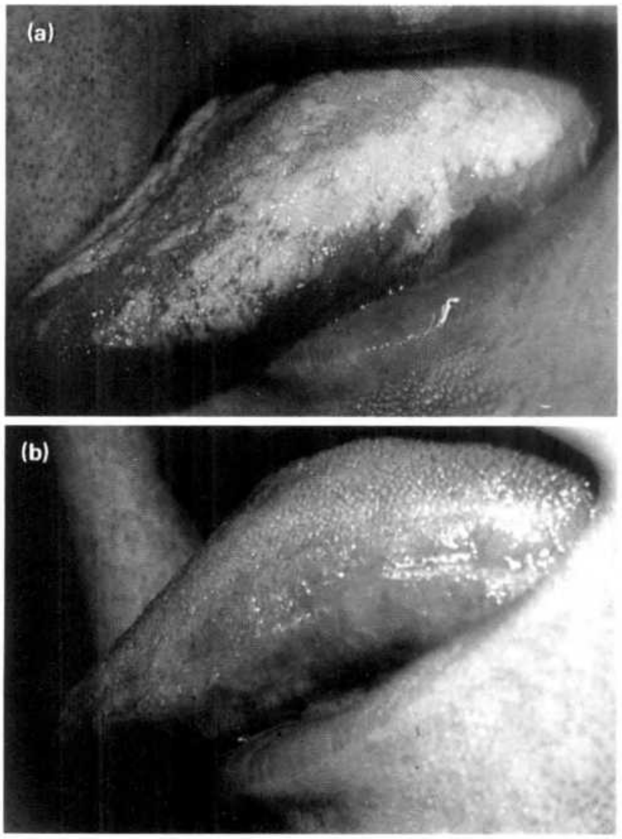

Figure 1. Lesions of oral hairy leukoplakia (a) at presentation, (b) after reducing the dose of systemic steroids.

Histology

A biopsy was taken from the lateral margin of the tongue and divided into three portions. One was fixed in Bouin's fixative and processed for routine histology and for immunohistochemistry. The second portion was snap

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frozen in liquid nitrogen and processed for immunofluorescence. The third portion was fixed in 2% glutaraldehyde and processed for transmission-electron microscopy.

Light microscopy

There was an irregularly thickened epithelium with parakeratosis and the superficial layer contained cells with eosinophilic cytoplasm (Fig. 2). In the upper epidermis there were koilocytoid cells, having a dark basophilic nucleus surrounded by clear vacuolated cytoplasm. In many of these cells there were nuclear basophilic inclusions of the Cowdry A type (Fig. 3). There were a few mononuclear cells around isolated capillary vessels in the upper dermis. Sections stained with PAS showed no evidence of *Candida*.

Immunohistochemistry

Immunohistochemistry using an antiserum with a very

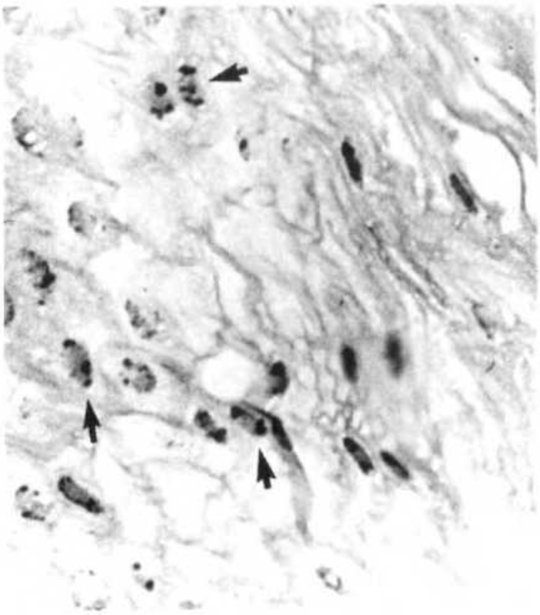

Figure 3. Intranuclear type A Cowdry inclusion (arrows) (haematoxylin and eosin, × 400).

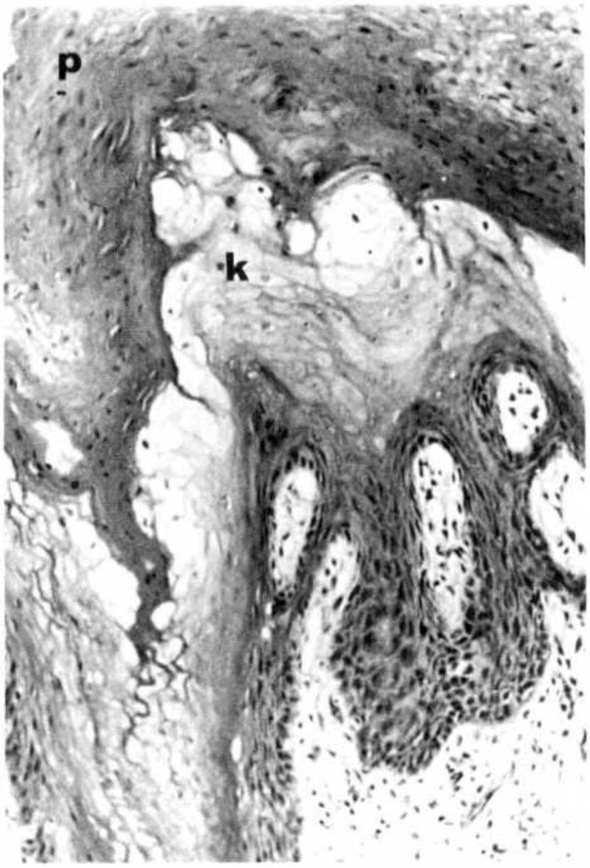

Figure 2. Histology of the lesion. Parakeratosis (p), irregular acanthosis with the presence of numerous koilocytoid cells (k) in the upper epidermis (haematoxylin and eosin, × 100).

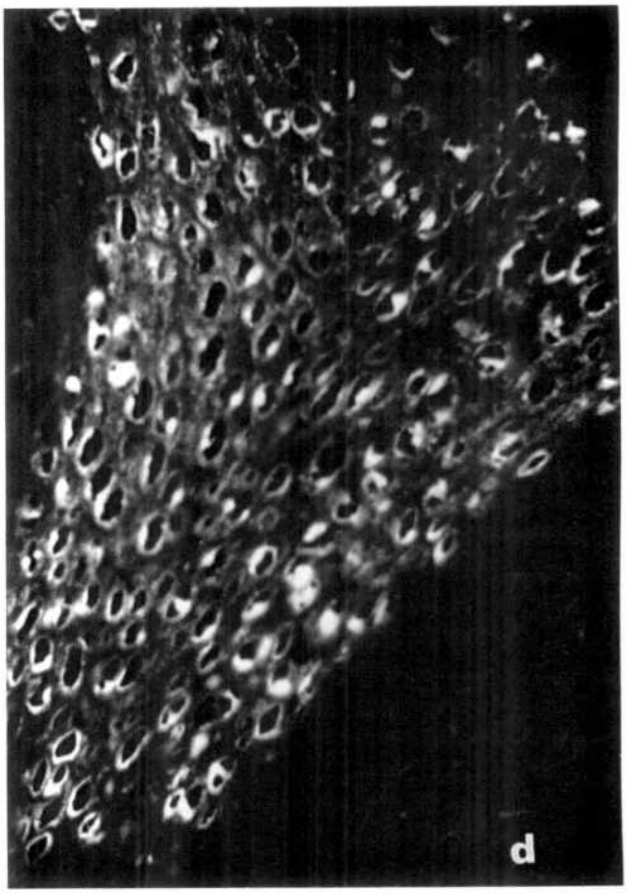

Figure 4. Fluorescence of the epithelium in OHL using anti-EBV-NA and -VCA antiserum (d, dermis) (× 250).

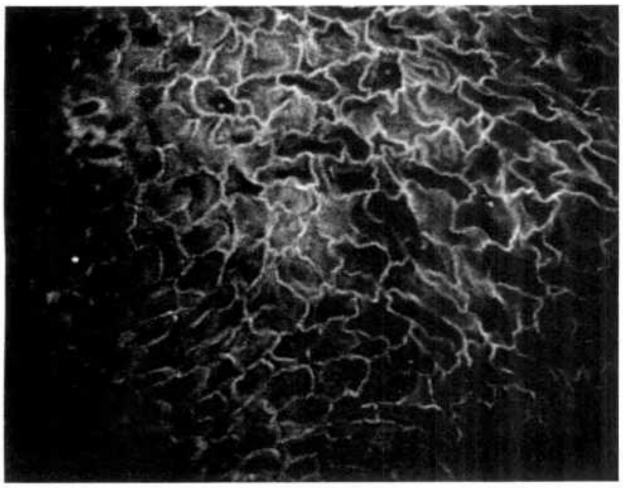

Figure 5. Indirect immunofluorescence showing expression of CD21 antigen (EBV receptor) on the plasma membrane of epithelial cells (× 210).

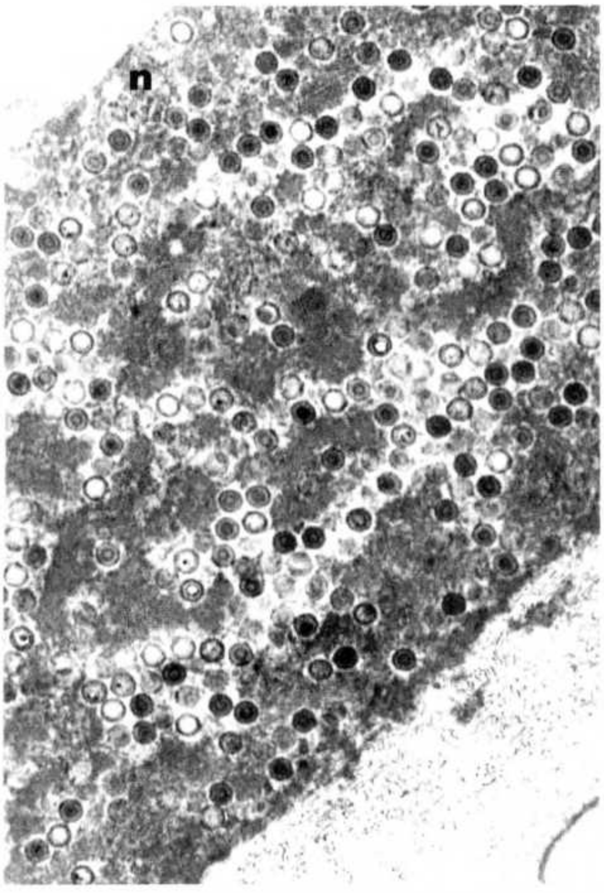

Figure 6. Electron micrograph showing abundant herpes-type virions at various stages of maturation within a keratinocyte nucleus (n) in the upper epidermis (× 20,000).

high titre of antibodies to EBV-NA and -VCA antigens showed a marked reactivity within the nucleus and cytoplasm of the keratinocytes in the upper layers of the epithelium (Fig. 4). Langerhans cells were seen in small numbers that stained positive with S100 and CD1a and HLA-DR in the lower part of the epidermis. Using OKB7 (Ortho, Raritan, NJ, U.S.A.) that recognizes the CD21 antigen of leucocyte differentiation that serves as the C3d (CR2) and EBV receptor, showed labelling of the plasma membrane of the keratinocytes (Fig. 5).

Electron microscopy

There was a heavy colonization of the upper epithelium with coccobacilli that were sometimes seen within the cytoplasm of the keratinocytes. The most striking finding was the presence of many herpes-type virions in various stages of maturation with capsids that measured 100–120 nm in diameter, either empty or with an electron-dense core (Fig. 6). These virions were to be seen within the nucleus and/or the cytoplasm of the keratinocytes as well as in the intercellular spaces.

Discussion

Oral hairy leukoplakia (OHL) is considered to be due to an opportunistic infection of EBV in the epithelial cells of the mucosa. The presence of EBV is considered to be an important diagnostic criterion for OHL.[2] There are however lesions that clinically and histologically resemble OHL (pseudo-oral hairy leukoplakia) but these are usually seen in non-immunocompromised individuals and do not contain EBV.[3] So far OHL has been observed almost exclusively in those patients infected with HIV.[4] It has been estimated that 48% of patients with OHL will progress to AIDS within 16 months following the development of the lesions.[5] Rarely, it has been claimed that OHL has been observed in HIV-negative individuals, in male homosexuals[1,6,7] and in a liver graft recipient.[8] However, no biopsy was performed in these cases and the diagnosis of OHL cannot therefore be confirmed. Lesions diagnosed as OHL have been reported in two allogeneic bone-marrow recipients although in these cases the presence of EBV was not investigated.[9] However, since 1988 there have been three cases of OHL that have been virologically confirmed and that have developed in HIV-negative renal and bone-marrow graft recipients who have been on immunosuppressive or cytostatic therapy.[10–12] Our patient who was HIV-negative, represents the fourth documented case of OHL and who was on immunosuppressive therapy.

The clinical, light and electron microscopic and the immunohistochemical findings in our case were indistinguishable from those seen in OHL associated with AIDS.[2,4,13,14] It appears that OHL is not specific for HIV infection but is seen when there is profound immunosuppression. In our patient the lesions of OHL appeared when immunosuppressive therapy was increased and regressed spontaneously when the treatment was reduced. Why OHL is seen more frequently in the course of HIV infection when compared to other immunodeficiency diseases is as yet unknown.

Acknowledgments

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References

1 Greenspan D, Greenspan J, Conant M *et al* Oral 'hairy' leukoplakia in male homosexuals: evidence for association with both papillomavirus and a herpes-group virus. *Lancet* 1984; **ii**: 831–4.
2 Greenspan J, Greenspan D. Oral hairy leukoplakia: diagnosis and management. *Oral Surg Oral Med Oral Pathol* 1989; **67**: 396–403.
3 Green T, Greenspan J, Greenspan D, De Souza Y. Oral lesions mimicking oral hairy leukoplakia: a diagnostic dilemma. *Oral Surg Oral Med Oral Pathol* 1989; **67**: 422–6.
4 Kanitakis J, Zambruno G, Marchand C *et al.* La leucoplasie orale chevelue du SIDA. Etude histologique et ultrastructurale de 8 cas. *Ann Dermatol Venereol* 1990; **117**: 345–53.
5 Greenspan D, Greenspan J, Hearst N *et al.* Relation of oral hairy leukoplakia to infection with the human immunodeficiency virus and the risk to developing AIDS. *J Infect Dis* 1987; **155**: 475–81
6 Matis WL, Triana A, Shapiro R *et al.* Dermatologic findings associated with human immunodeficiency virus infection. *J Am Acad Dermatol* 1987; **17**: 746–51.
7 Melnick S, Engel D, Truelove E. Oral disease and HIV infection. *IVth International Conference on AIDS*, Stockholm, 12–16 June 1988.
8 Reggiani M, Pauluzzi P. Hairy leukoplakia in a liver transplant patient. *Acta Derm Venereol (Stockh)* 1990; **70**: 87–8.
9 Epstein J, Priddy R, Sherlock C. Hairy leukoplakia-like lesions in immunosuppressed patients following bone-marrow transplantation. *Transplantation* 1988; **46**: 462–4.
10 Itin P, Rufli T, Rudlinger R *et al.* Oral hairy leukoplakia in HIV-negative renal transplant patient: a marker for immunosuppression? *Dermatologica* 1988; **177**: 126–8.
11 Syrjanen S, Laine P, Happonen R, Niemela M. Oral hairy leukoplakia is not a specific sign of HIV-infection but related to immunosuppression in general *J Oral Pathol Med* 1989; **18**: 28–31.
12 Greenspan D, Greenspan J, De Souza Y, Ungar A. Oral hairy leukoplakia in an HIV-negative renal transplant recipient. *J Oral Pathol Med* 1989, **18**: 32–4.
13 Fowler C, Reed K, Brannon R. Intranuclear inclusions correlate with the ultrastructural detection of herpes-type virions in oral hairy leukoplakia. *Am J Surg Pathol* 1989; **13**: 114–9.
14 Daniels T, Greenspan D, Greenspan J *et al.* Absence of Langerhans cells in oral hairy leukoplakia, an AIDS-associated lesion. *J Invest Dermatol* 1987; **89**: 178–82.

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Is erythrokeratoderma one disorder? A clinical and ultrastructural study of two siblings

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Summary Two sisters with erythrokeratoderma are described. In the younger sister the clinical appearance corresponded to erythrokeratoderma variabilis (EKV), whereas in the older sister it corresponded to progressive symmetrical erythrokeratoderma (PSEK). Ultrastructural findings in both cases were identical. We suggest that EKV and PSEK are different manifestations of a single condition.

The term erythrokeratoderma variabilis (EKV) was first used by Mendes da Costa in 1925 to describe a mother and daughter with relatively fixed patches of hyperkeratosis and erythematous areas 'characterized by capriciously formed outlines, like the boundary lines of sea-coasts on maps'.[1] In contrast to the hyperkeratotic areas, the erythematous areas moved from hour to hour. EKV is inherited in most cases as an autosomal dominant condition[2,3] and the largest pedigree is that investigated by Noordhoek.[4] Genetic linkage with the rhesus blood group has recently been shown.[5] The condition tends to present at birth or during the first year, but may begin later in childhood or in early adulthood. Lesions preferentially affect the face, buttocks, and extensor surfaces of the extremities. Palmoplantar keratoderma occurs in up to half the cases but structures such as hair, nails and teeth are not affected.

The original description of progressive symmetrical erythrokeratoderma (PSEK) was probably that of Darier,[6] although the name by which the condition is now known was given by Gottron in 1922.[7] It is also inherited as an autosomal dominant condition, with incomplete penetrance and variable expressivity. Hyperkeratotic plaques are distributed symmetrically over the body, particularly on the extremities and buttocks but also on the face.[8–12] Again, palmoplantar keratoderma is often present. Epidermal cell proliferation is reportedly increased.[13]

The distribution of hyperkeratotic lesions in both conditions is thus very similar and the main distinction between EKV and PSEK is the presence of variable erythema in EKV. There is no exact definition of either condition. However, some reports of EKV do not mention the erythematous component at all,[14] whereas many reports of PSEK clearly state that the hyperkeratotic plaques arise on an erythematous base.

A limited number of ultrastructural studies have been performed in the erythrokeratodermas. In EKV, two studies noted clumped perinuclear tonofilaments and subnormal numbers of membrane coating granules (MCGs, keratinosomes) within the stratum granulosum.[2,15] In another patient MCG numbers were normal.[16] In PSEK, one study noted marked swelling of mitochondria in the stratum granulosum, with lipid-like vacuoles in corneocytes.[8]

Case reports

Case 1 (J.S., aged 5 years)

This girl had well-demarcated erythema of the soles at birth. When she was 17 months old she developed a recurrent red eruption on the face, trunk and extremities. These expanding, migrating annular lesions lasted from several hours to 2 days, often appearing at times of marked environmental temperature change or when febrile. At the age of 3 years she developed scaly, slightly pigmented patches on the cheeks and ears, pigmented and hyperkeratotic plaques distributed symmetrically on the posterior thighs (Fig. 1), and palmoplantar keratoderma. The plaques may become temporarily more erythematous at times. The clinical diagnosis was EKV.

Case 2 (S.S., aged 8 years)

This girl, the older sister of J.S., had no skin problems before the age of 6 years. She then developed pigmented, scaly plaques on the cheeks and chin (Fig. 2), with erythema and peeling of the pinnae. Pigmented, scaly

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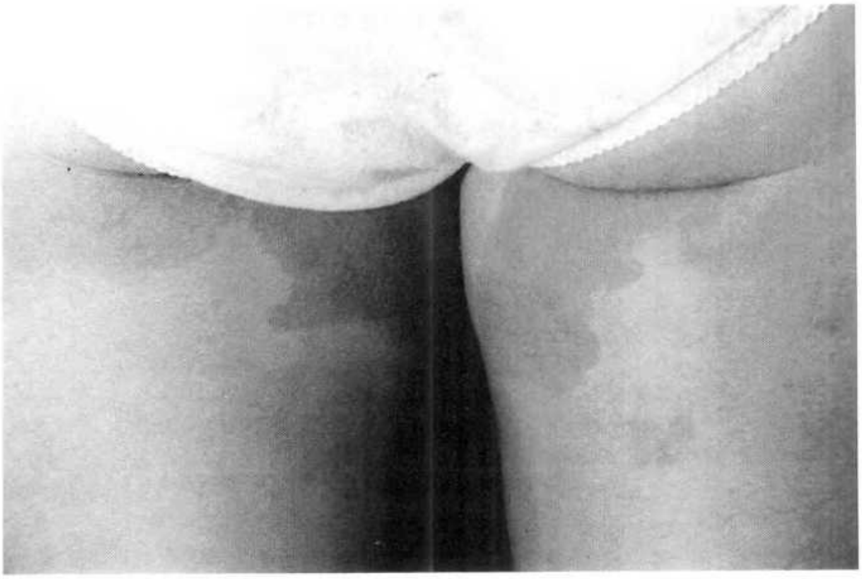
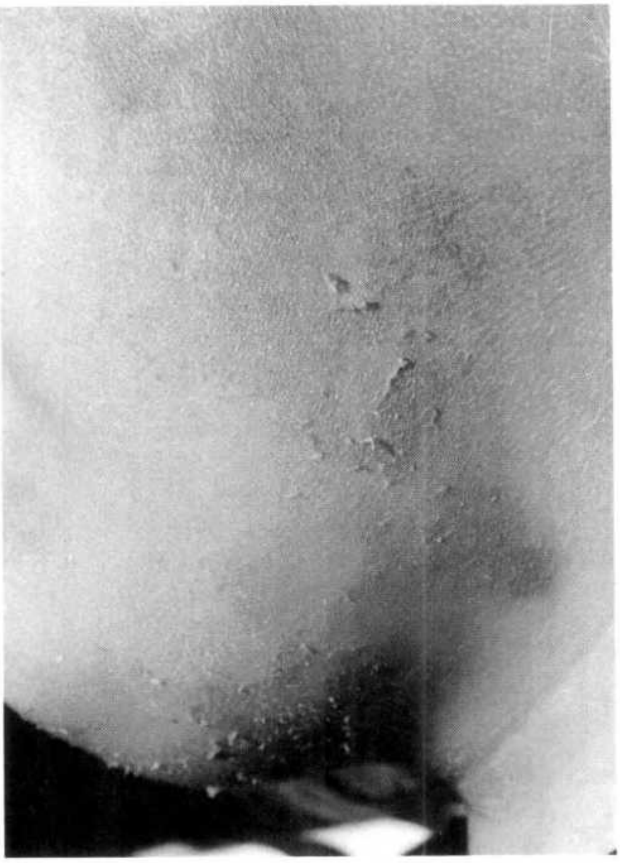


Figure 1. Case 1 (J.S.) Hyperpigmented and hyperkeratotic plaques distributed symmetrically on the posterior thighs.



plaques were also present in a remarkably symmetrical distribution on the medial thighs, knees and elbows, and there was keratoderma of the soles and palms. There were no migrating areas of erythema but from the age of 8 years the fixed patches and the skin around them often became redder in extremes of environmental temperature. The diagnosis in this child was more that of PSEK than EKV, which had been diagnosed in her sister.

There was neither a family history of erythrokeratoderma nor were the parents consanguineous. Both children were treated with preparations including 5% lactic acid ointment, Sential® cream (0·5% hydrocortisone + 4% urea + 4% sodium chloride), Calmurid® cream (10% urea + 5% lactic acid), and an emollient cream kindly supplied by Unilever, all with some improvement.

Light microscopy

S.S. (Case 2) had a skin biopsy taken from a hyperkeratotic plaque. Light microscopy was not diagnostic, showing only orthohyperkeratosis with basket-weave keratin, a well-preserved granular cell layer, some acanthosis, and a sparse upper dermal lymphoreticular infiltrate.

Electron microscopy

Technique. In both children samples for electron microscopy were removed from hyperkeratotic plaques and cut into pieces 1 mm^3. Specimens were then fixed for 1 h with 2% formaldehyde and 2·5% glutaraldehyde in 0·1 M cacodylate buffer (pH 7·2) containing 5% sucrose and 0·05% calcium chloride, post-fixed with 1% osmium tetroxide, dehydrated, and embedded in Epon. To enhance specifically the contrast of membrane-coating granules (MCGs) some additional specimens were secondarily fixed with imidazole-buffered osmium tetroxide.[17] Ultrathin sections (60 nm) stained with 5% uranyl acetate and Reynolds lead citrate were viewed in a Philips EM 300 transmission-electron microscope.[18]

Results. Ultrastructural observations revealed no obvious differences between the samples from each child. The only apparent subcellular changes occurred in the stratum granulosum. In contrast to normal control epidermis (Fig. 3a) a prominent tonofilament/keratohyalin granule (KHG) complex enveloped a clear cytoplasmic halo around a nucleus which was often crenellated (Figs 4a and 5a). No other differences were detected. In particular, no mitochondrial swelling was observed at any level in the epidermis and no lipid droplets were seen in the stratum corneum. Furthermore, numerous MCGs were seen in both specimens (Figs 4b and 5b), as well as in control tissue (Fig. 3b). MCGs retained their characteristic lamellar appearance and, as with controls, extruded their contents extracellularly in the stratum granulosum.

Discussion

The changes seen by light microscopy in EKV and PSEK comprise orthohyperkeratosis, a granular cell layer of two to four cells, irregular acanthosis, suprapapillary thinning, papillomatosis and a variable dermal inflammatory infiltrate: there are no consistent features that permit a reliable distinction between the two conditions, although perinuclear vacuolization and parakeratosis have been described in PSEK.[8,9] In one patient with EKV dyskeratotic Darier-like cells containing clumped perinuclear tonofilaments were found below the stratum corneum in normal and involved skin.[15]

With regard to ultrastructural abnormalities, two previous reports suggest that MCG (keratinosome) numbers are subnormal in the spinous and/or granular cell layers of the epidermis in EKV.[2,15] The number of MCGs in one patient was restored to normal after isotretinoin therapy.[15] This finding is not apparently specific for EKV: MCGs were also decreased in one case of the Curth–Macklin type of ichthyosis hystrix.[19] In another case of EKV, MCG numbers were normal.[16]

There is only one ultrastructural study of PSEK.[8] MCGs were numerous but granular cells contained

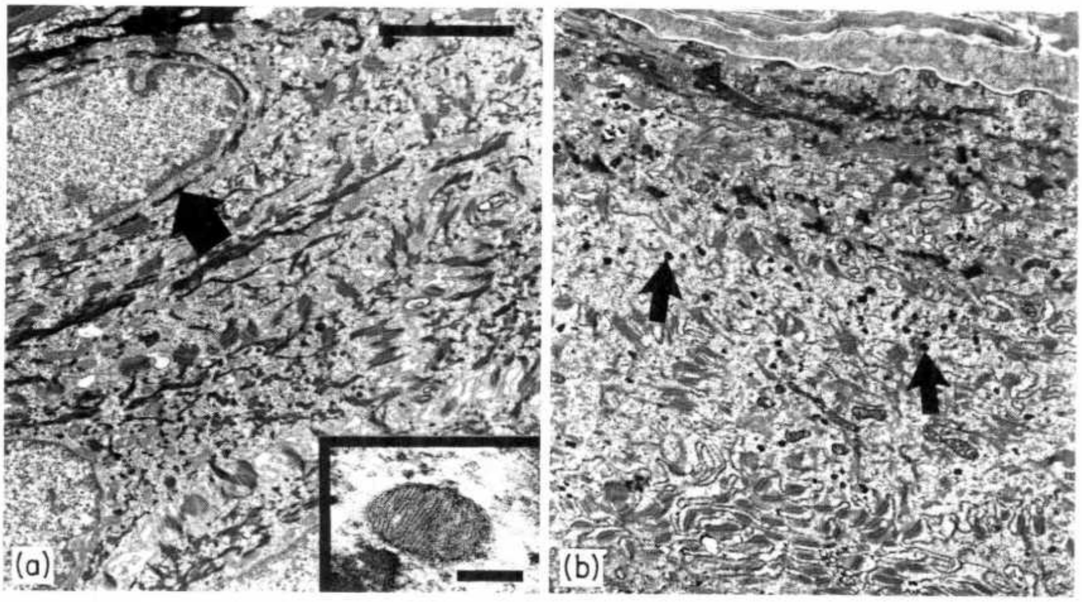


Figure 3. Normal epidermis displaying relatively discrete tonofilament/KHG complexes (a, arrow). Following conventional fixation MCGs retain their characteristic lamellar structure (a, inset) but are difficult to detect at low magnification (a). However, MCGs are evident after secondary fixation with imidazole-buffered osmium tetroxide (b, arrows). (a and b: bar = 2·0 μm; × 8,360. Inset: bar = 0·1 μm; × 86,350).

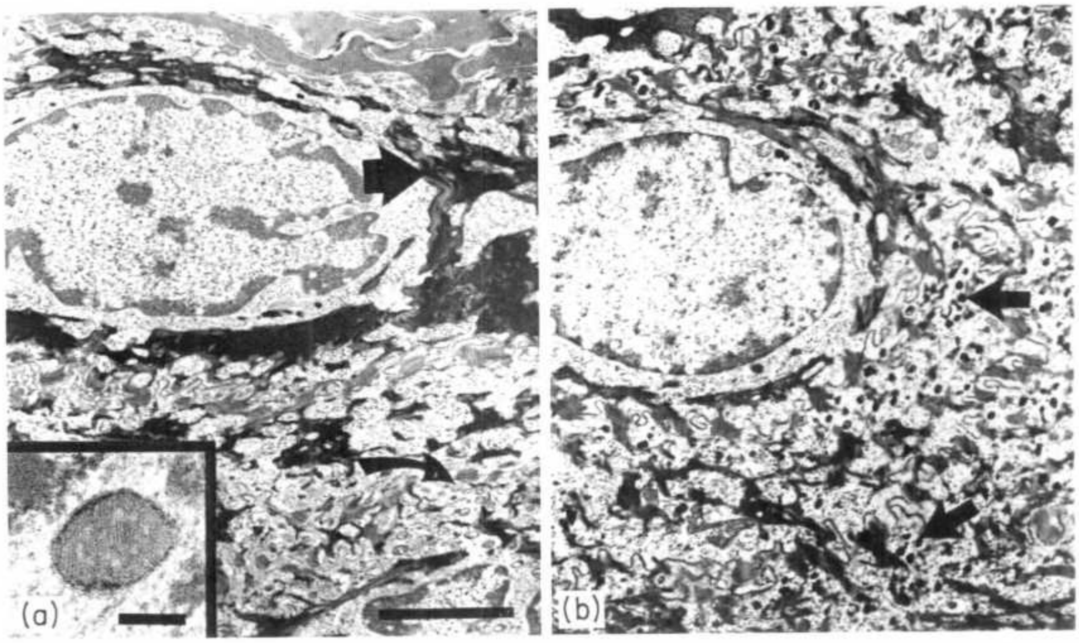


Figure 4. Epidermis from Case 1 (J.S., clinically with EKV) displaying a crenellated nucleus surrounded by a dense tonofilament/KHG complex (a). In the conventionally fixed specimen, MCGs are just discernible at low magnification (a, curved arrow) but their lamellar substructure is obvious at higher magnification (a, inset). Following imidazole-buffered fixation MCGs are clearly seen, even at low magnification (b, arrows). (a and b: bar = 2·0 μm; × 8,360. Inset: bar = 0·1 μm; × 86,350).

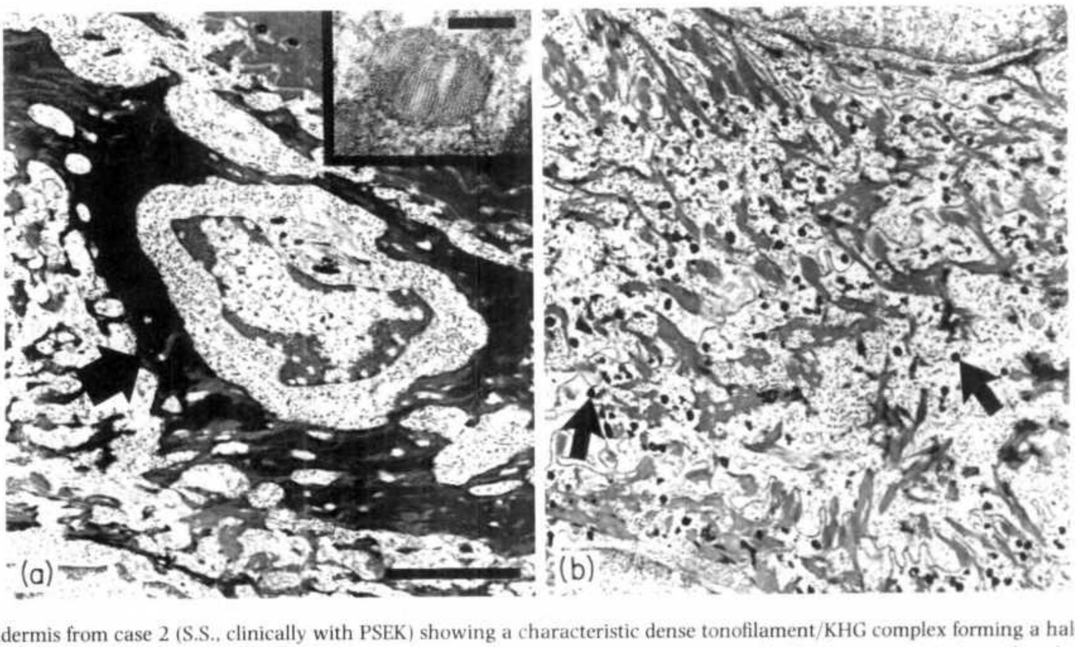


Figure 5. Epidermis from case 2 (S.S., clinically with PSEK) showing a characteristic dense tonofilament/KHG complex forming a halo around the nucleus (a, arrow). At high magnification the lamellar structure of MCGs is evident in conventionally fixed specimens (a, inset) but these organelles are difficult to detect at low magnification (a), unless exposed to imidazole-buffered osmium tetroxide (b, arrows). (a and b: bar = 2·0 μm; × 8,360. Inset: bar = 0·1 μm; × 86,350).

swollen, disrupted mitochondria, and keratinocytes contained lipid-like vacuoles. These abnormalities were less marked after etretinate.

MCGs are frequently difficult to visualize in conventionally fixed tissue. This may explain the discrepancies in MCG numbers reported for EKV by different groups.[2,15,16] However, in our cases we noted MCGs that were morphologically and numerically normal in specimens from both children following specific enhancement of MCGs by imidazole-buffered osmium tetroxide. Mitochondria were normal.

The children we describe had no other abnormalities and, indeed, associated defects are rare. One French-Canadian family with EKV showed neurological abnormalities (decreased tendon reflexes, nystagmus, dysarthria and gait ataxia) in 25 members over five generations.[20] One patient developed a progressive encephalopathy including cerebellar ataxia,[21] and a syndrome of EKV with growth retardation, perceptive deafness, and peripheral neuropathy has been reported.[22] PSEK has been described with sensory hearing loss[23] and with pachyonychia.[24]

The erythrokeratodermas are usually resistant to topical therapy although systemic etretinate or isotretinoin may be effective.[8,15,25–27] Oral retinoids were not tried in our cases.

We suggest that EKV and PSEK may be different manifestations of the same inherited condition. The occurrence of each type of erythrokeratoderma in two siblings has not been described previously, and given the exceptional rarity of the conditions it is unlikely to be due to chance. The biopsies from each child were ultrastructurally indistinguishable and no abnormality was found in the numbers or morphology of the MCGs and there was no abnormality of the mitochondria. However, a prominent tonofilament/keratohyalin granule complex was noted around a nucleus which was often crenellated. This abnormality was present in both patients.

Acknowledgment

We thank Dr J.Kellett for referring these children to one of us (J.L.V.).

References

1 Mendes da Costa S. Erythro- et keratodermia variabilis in a mother and daughter. *Acta Derm Venereol (Stockh)* 1925; **6**: 300–4.

2 Vandersteen PR, Muller SA. Erythrokeratodermia variabilis. An enzyme histochemical and ultrastructural study. *Arch Dermatol* 1971; **103**: 362–70.

3 Gewirtzman GB, Winkler NW, Dobson RL. Erythrokeratodermia variabilis. *Arch Dermatol* 1978; **114**: 259–61.

4 Noordhoek FJ. *Over Erythro- et Keratodermia Variabilis*. Schiedam, West Germany: Drukherij de Eendracht, 1950.

5 van der Schroeff JG, van Leeuwen-Cornelisse I, van Haeringen A, Went LN. Further evidence for localization of the gene of erythrokeratodermia variabilis. *Hum Genet* 1988; **80**: 97–8.

6 Darier J. Erythrokératodermie verruqueuse en nappes, symétrique et progressive. *Bull Soc Fr Dermatol Syphiligr* 1911; **22**: 252–64.

7 Gottron H. Erythrokeratodermia progressiva symmetrica. *Zentbl Haut-Geschl-Krankh* 1922; **4**: 493.

8 Nazzaro V, Blanchet-Bardon C. Progressive symmetric erythrokeratodermia. *Arch Dermatol* 1986; **122**: 434–40.

9 Ruiz-Maldonado R, Tamayo L, del Castillo V, Lozoya I. Erythrokeratodermia progressiva symmetrica. *Dermatologica* 1982, **164**: 133–41.

10 Rodriguez-Pichardo A, Garcia-Bravo B, Sanchez-Pedreno P, Camarcho-Martinez F. Progressive symmetric erythrokeratodermia. *J Am Acad Dermatol* 1988; **19**: 129–30.

11 Hopsu-Havu VK, Peltonen L. Erythrokeratodermia congenitalis progressiva symmetrica (Gottron). *Dermatologica* 1970; **141**: 321–8.

12 Nir M, Tanzer F. Progressive symmetric erythrokeratoderma. *Dermatologica* 1978; **156**: 268–73.

13 Hopsu-Havu VK, Tuohimata P. Erythrokeratodermia congenitalis progressiva symmetrica (Gottron). II: An analysis of kinetics of epidermal cell proliferation. *Dermatologica* 1971; **142**: 137–44.

14 Schellander FG, Fritsch PO. Variable erythrokeratoderma. *Arch Dermatol* 1969; **100**: 744–8.

15 Rappaport IP, Goldes JA, Goltz RW. Erythrokeratodermia variabilis treated with isotretinoin. *Arch Dermatol* 1986; **122**: 441–5.

16 Jurecka W. Erythrokeratodermia variabilis. *Arch Dermatol* 1986, **122**: 1356.

17 Angermuller S, Fahimi HD. Imidazole-buffered osmium tetroxide: an excellent stain for visualisation of lipids in transmission electron microscopy *Histochem J* 1982; **14**: 823–5.

18 Reynolds ES. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J Cell Biol* 1963; **17**: 208–12

19 Kanerva L, Karvonen J, Oikarinen A *et al.* Ichthyosis hystrix (Curth-Macklin). *Arch Dermatol* 1984; **120**: 1218–23.

20 Giroux J-M, Barbeau A. Erythrokeratodermia with ataxia. *Arch Dermatol* 1972; **106**: 183–8.

21 Maradian MH, Kouchanfar A. Syndrome familial associant une erythrokératodermie variable et une encephalopathie progressive. *Arch Fr Pediatr* 1986; **43**: 535.

22 Beare JM, Nevin NC, Froggatt P *et al.* Atypical erythrokeratodermia with deafness, physical retardation and peripheral neuropathy. *Br J Dermatol* 1972; **87**: 308–14.

23 Lamprecht A, Goecke T, Anton-Lamprecht I, Kuster W. Progressive erythrokeratodermia and cochlear hearing impairment. *Int J Pediatr Otorhinolaryngol* 1988; **15**: 279–89.

24 Kuchmeister B, Schaeg G, Kuhlwein A. Erythrokeratodermia congenitalis progressiva symmetrica Gottron mit atypischem Befall der Nagel im Sinne einer Pachyonychie. *Z Hautkr* 1983; **58**: 621–32.

25 Fixler ZC. Treatment of erythrokeratodermia variabilis with oral synthetic retinoids. *Cutis* 1980; **25**: 300–4.

26 Tamayo L, Ruiz-Maldonado R. Oral retinoid (Ro 10-9359) in children with lamellar ichthyosis, epidermolytic hyperkeratosis and symmetrical progressive erythrokeratoderma. *Dermatologica* 1980; **161**: 305–14.

27 Peck GL, Yoder FW. Treatment of lamellar ichthyosis and other keratinizing dermatoses with an oral synthetic retinoid. *Lancet* 1976; **ii**: 1172.

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Congenital malignant melanoma

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Summary A case is reported of congenital malignant melanoma that occurred in the absence of maternal melanoma or a congenital giant naevus. The patient had the melanoma on the left thigh excised 54 days after birth, but later died at the age of 18 months with multiple metastases.

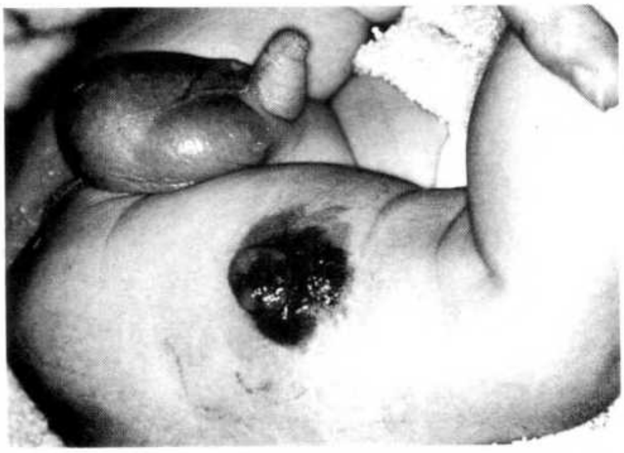

Figure 1. Lesion on left thigh at 40 days.

Congenital malignant melanoma of the skin represents a small subset of melanomas in childhood.[1–3] The tumour can be acquired from the mother transplacentally or may arise in a giant congenital melanocytic naevus. Non-metastasizing pigmented tumours in children can be microscopically indistinguishable from melanoma and have often been misdiagnosed.[1] We report a case of congenital malignant melanoma with metastases that occurred in the absence of melanoma in the mother and there was no giant congenital naevus in the child.

Case report

A Japanese boy was born in March 1987 after 37 weeks'

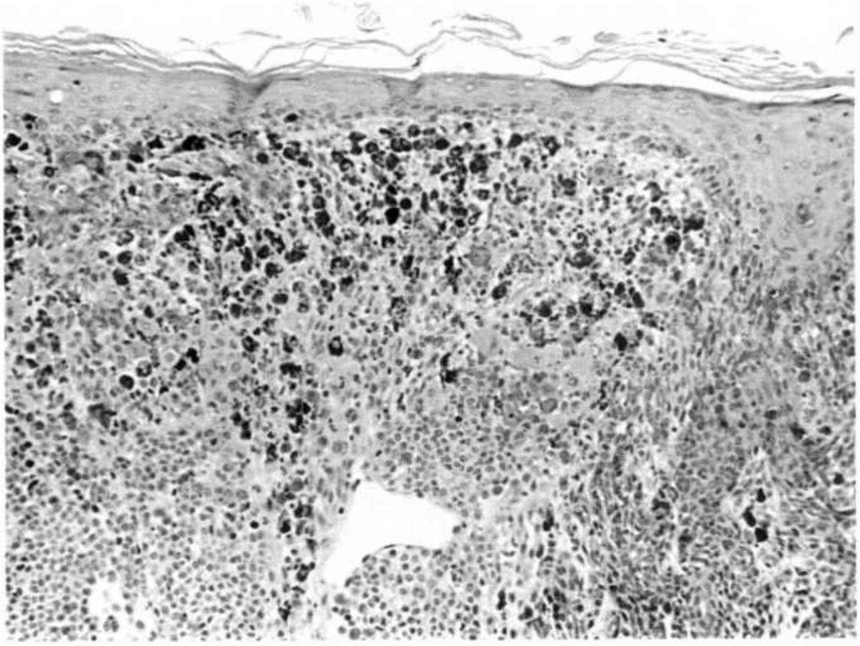

Figure 2. Atypical melanocytes evident throughout the dermis (haematoxylin and eosin, × 40).

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Table 1. Congenital metastatic malignant melanoma

Author (ref)	Patient age at diagnosis	Sex	Location of primary lesion	Location of metastasis	Survival after diagnosis	
					Death	Alive
Coe[4]	3 months	F	Scalp	Regional node	5 months	
Truax and Page[5]	9 years	F	Right knee	Regional node		6 years
McWhorter and Woolner[6]	9 years	F	Ear	Local recurrence, regional node	1 year	
McWhorter and Woolner[6]	10 years	M	Cheek	Regional node, femur	6 years	
Brandt[7]	16 months	M	Trunk	Widespread metastasis later noted	7 months	
Myhre[8]	14 years	M	Back	Local recurrence, regional node	3 years	
Myhre[8]	10 years	F	Back	Local recurrence, regional nodes	2 5 years	
Lyall[9]	12 months	M	Tip of right middle finger	Regional nodes		2·5 years
Oldhoff and Koudstaal[10]	Shortly after birth	M	Right thigh	Local recurrence		10 years
Lerman *et al.*[11]	12 years	F	Right orbital tissue	Local recurrence	17 months	
Conu *et al.*[12]	7 days	F	Left foot	Regional node	Lost to follow-up	
Stromberg[13]	4 months	M	Left mastoid process	Regional node		18 years
Present case	2 months	M	Left thigh	Lung, bone, liver, skin	1·5 years	

gestation and together with a biovular twin. His mother and twin sister were normal. At birth it was noted that there was a dark black macule that measured 22 × 22 mm on the left thigh with a central red–black nodule that measured 7 × 7 × 4 mm. Forty days after birth the macule and nodule had increased in size to 25 × 25 mm and 15 × 16 × 7 mm, respectively (Fig. 1). A biopsy showed the tumour to be composed of naevus cells extending to the subcutaneous fat. The intradermal neoplastic cells were relatively small and uniform, but had hyperchromatic nuclei and there were frequent mitotic figures (Fig. 2). A diagnosis of malignant melanoma was made. Fifty-four days after birth the tumour was excised.

By the age of 13 months there were multiple metastases in the lung, bone, liver and skin and chemotherapy with dimethyl-triazeno-imidazole carboxamide (DTIC), methyl CCNU and vincristine were instituted. However, the child died at the age of 18 months with disseminated intravascular coagulation.

Discussion

Melanoma occurring in childhood is rare and accounts for only 0·4% of all cases.[1] In a comprehensive review of the literature[1] childhood malignant melanoma was divided into three types: congenital transplacentally acquired malignant melanoma (type I), malignant with onset before puberty (type II) and malignant melanoma before puberty arising in a giant congenital melanocytic naevus (type III). Similar cases to our own of congenital metastatic malignant melanoma have been reported[4–13] (Table 1). Of the reported patients that includes our case there were seven boys and six girls and all had clinical and histological evidence of regional or distant metastases before adolescence. Eight died from metastatic disease and one was lost to follow-up. The remaining four patients were free of disease for more than 2 years following treatment.

References

1 Trozak DJ, Rowland WD, Hu F. Metastatic malignant melanoma in prepubertal children. *Pediatrics* 1975, 55: 191–204.

2 Pratt CB, Palmer MK, Thatcher N, Crowther D. Malignant melanoma in children and adolescents. *Cancer* 1981; 47: 392–7.

3 Prose NS, Laude TA, Heilman ER, Coren C. Congenital malignant melanoma. *Pediatrics* 1987; 79: 967–70.

4 Coe HE. Malignant pigmented mole in an infant. *Northwest Med* 1925; 24: 181–2.

5 Truax KF, Page HG. Prepubertal malignant melanoma. *Ann Surg* 1953; 137: 255–60.

6 McWhorter HE, Woolner LB. Pigmented nevi, juvenile melanomas, and malignant melanomas in children. *Cancer* 1954; **7**: 564–85.
7 Brandt G. Melanoma of the skin. *Ann Chir Gynaecol Fenn* 1956; **45**(Suppl. 3): 97–114.
8 Myhre E. Malignant melanomas in children. *Acta Pathol Microbiol Scand* 1963; **59**: 184–8.
9 Lyall D. Malignant melanoma in infancy. *JAMA* 1967, **202**: 1153.
10 Oldhoff J, Koudstaal J. Congenital papillomatous malignant melanoma of the skin. *Cancer* 1968; **21**: 1193–7.
11 Lerman RI, Murray D, O'Hara JM *et al.* Malignant melanoma of childhood. A clinicopathologic study and a report of 12 cases. *Cancer* 1970; **25**: 436–49.
12 Conu A, Nicolescu F, Popescu G. Malignant melanoma developing in intrauterine life. *Rom Med Rev* 1971; **15**: 41–42.
13 Stromberg BV. Malignant melanoma in children. *J Pediatr Surg* 1979; **14**: 465–467.

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Congenital hypertrichosis lanuginosa and congenital glaucoma

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Summary An infant with congenital hypertrichosis lanuginosa (CHL) and congenital glaucoma is presented. Both conditions are rare and have not been previously reported to occur in association.

Case report

This male infant was born in April 1990, after a normal pregnancy and delivery. His mother had not taken any medication or supplementary preparations during the pregnancy. He had a birth weight of 2·99 kg and normal Apgar scores.

On examination he had a profuse growth of long blond, silky hair covering most of his body (Fig. 1), including the face, ears, trunk, genitalia and limbs, with sparing of the palms, soles and mucous membranes. It was most dense around the external auditory canals and over the sacrum. The scalp, eyebrow and eyelash hair was unremarkable, being slightly darker and coarser and blending with the silky hair at the margins of the hairlines. In addition, he had a clouded right cornea and there was a suspicion of left-sided microphthalmos. There were no other congenital defects and his mouth and nails were normal. The length, weight and head circumference were around the 50th percentiles. A general examination, including assessment of respiratory, cardiovascular and neurological systems, was normal.

He was the second infant of healthy, unrelated Caucasian parents. His sibling, a 2½-year-old male, was well; both the father and brother were reported to have been hairy at birth, although this was not considered abnormal. Recent examination of both showed only a mild increase in body hair of normal appearance. There was no history of eye disease in either of his parents families.

Progress

There were no immediate neonatal problems. He was vigorous and fed well. At the age of 10 days he was examined under ketamine general anaesthetic as it does not spuriously lower the intraocular pressure, as may halothane. Abnormalities were found in both eyes. In the right eye the cornea was oedematous. At the pupillary margin the posterior pigmented layer of the iris was turned outward (ectropion uveae) with ovalling of the pupil and adhesions of the peripheral iris to the back of the cornea (anterior synechiae). The intraocular pressure was raised at 27 mmHg, as measured by applanation tonometry. The corneal diameter was 11 mm in the right eye and 10 mm in the left eye (Fig. 2).

In the left eye the iris was also abnormal with ectropion uveae (Fig. 3) and obvious transillumination when a light was shone in the eye. The posterior termination of the basement membrane of the corneal endothelium was visible (posterior embryotoxon), a feature which is found in association with maldevelopment of the anterior segment of the eye. However, the cornea was clear and the intraocular pressure was normal at 17 mmHg.

Management

Full general anaesthesia was induced and a trabeculotomy was performed. This involved making a small incision externally and passing a probe through the canal of Schlemm, breaking through the abnormal trabecular meshwork into the eye. A thick fibrous band of tissue was encountered over the trabecular meshwork and was divided.

He was examined again under general anaesthesia at the age of 1 month and again at 3 months, when the right eye was found to have responded well to treatment. The intraocular pressure was 17 mmHg and the cornea had cleared considerably. Iris transillumination was also present in this eye. Examination of the anterior chamber

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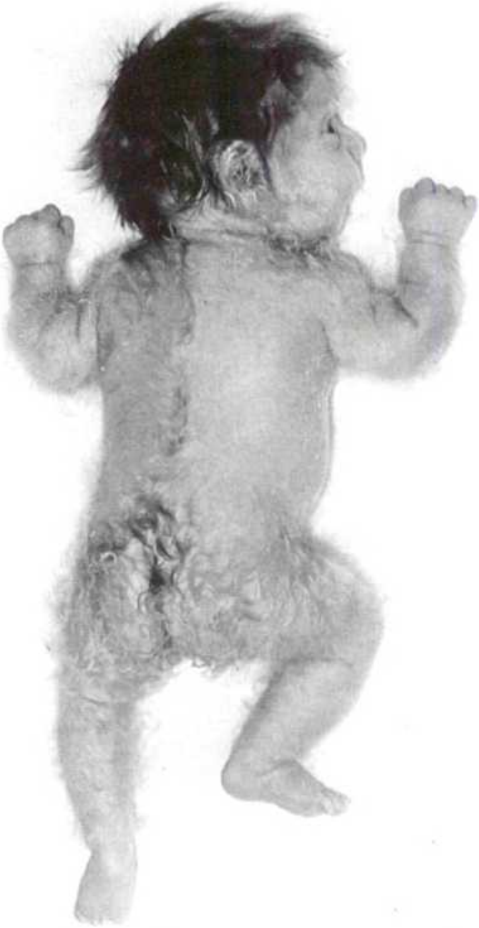

Figure 1. Generalized hypertrichosis lanuginosa at 6 weeks of age.

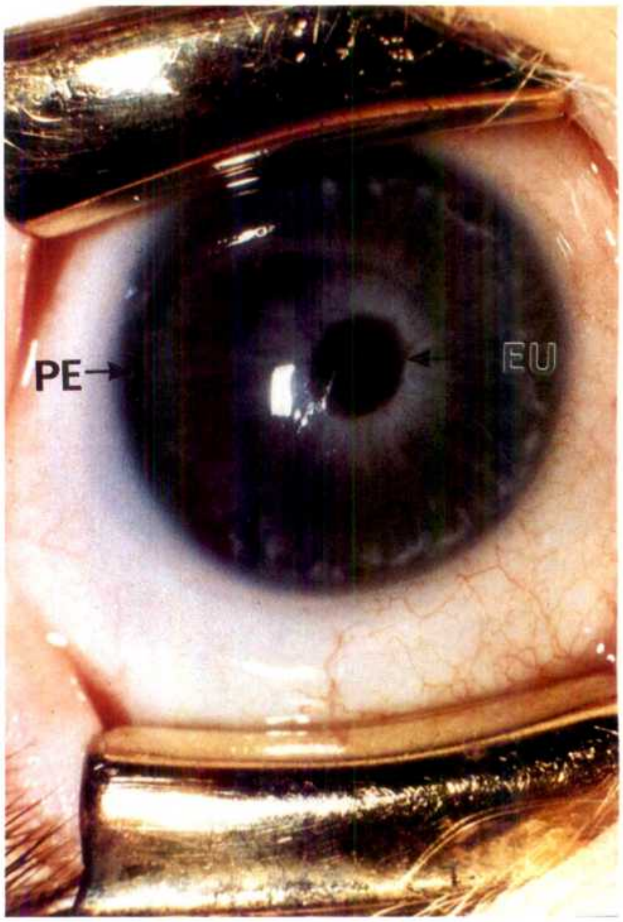

Figure 3. Left eye with normal intraocular pressure but showing abnormal features such as ectropion uveae (EU) and posterior embryotoxon (PE).

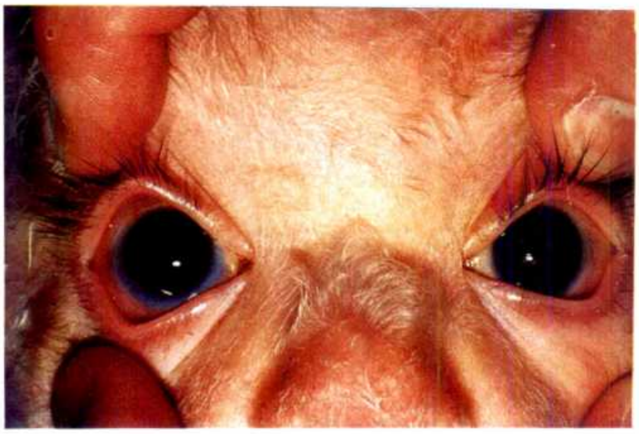

Figure 2. Right eye with raised intraocular pressure is larger than the left eye.

angle using a contact lens (gonioscopy) revealed an abnormally developed angle with an open cleft where the trabeculotomy had been performed. Patching of the left eye was commenced to reduce the risk of amblyopia in the right eye.

Over the ensuing 6 months he fed well, gained weight appropriately and developed normally. The hypertrichosis persisted, but was less prominent over the face and dorsa of the hands and feet. Regular trimming of the periaural, perinasal and perianal hair was performed to prevent matting.

Discussion

Hypertrichosis is the excessive growth of body hair in a normal distribution for the patient's race, age and sex. It differs from hirsutism which is the excessive growth of coarse terminal hair in a female, but distributed in a male sexual pattern. CHL, also known as hypertrichosis universalis lanuginosa, is a rare genetic disorder which has been reported to occur in all races. The first description of the condition appeared in the German literature in the 1870s[1] and by 1969, 32 cases had been reported. However, many of these individuals differed in time of onset, evolution and distribution of the hypertrichosis, and some may have been related to genetic syndromes or maternal illness.

In contrast to other causes of hypertrichosis, newborn infants with CHL have blond, silky lanugo hair affecting all areas except for those sites without hair follicles such as the palms and soles. The lanugo hair may reach a length of 10 cm and blends with the darker terminal hairs at the scalp and eyebrows. A common feature is a prominent ridged concentration of hair over the dorsal and lumbosacral spines and matted whorls around the pinnae, preauricular areas and sacrum.

A case report in 1970 by Beighton[2] of a boy with CHL, born in 1962, described him as having an excess growth of thick, dark scalp and eyebrow hair at birth, but noted that generalized hypertrichosis only became apparent during infancy, so that by the age of 4 he was covered with fine lanugo-like hair. In late childhood truncal and limb hypertrichosis diminished but shaving and depilatory creams were needed to reduce facial hairs. His father was reported to have been unduly hairy in infancy but in adult life had only focal increase in eyebrow and eyelash hair. In a separate report by Suskind[3] in 1971, of what appears to be the same patient, the infant is said to have had a striking amount of lanugo hair over the face and forearms and a progressive dense growth of golden yellow hair over the trunk, which reached its maximum at 2 years. Freire-Maia *et al.*[4] described a mother and son with hypertrichosis lanuginosa where the mother's disorder was more suggestive of hirsutism. The 16-year-old son had severe hypertrichosis and photophobia of unknown cause and he also had pubertal delay and short stature. There is no record of their appearance at birth or throughout childhood. Partridge[5] reported a female infant who had extensive CHL at birth and whose appearance was similar to our patient. She had a single precocious tooth and developed pyloric stenosis at 5 weeks of age. Her father and other members of his family were said to have had hypertrichosis and precocious teeth at birth. The index case was shaved regularly up to the age of 9 months, when it was apparent that the body was spontaneously shedding.

These and other reports suggest that CHL is transmitted as an autosomal dominant trait, but it is difficult to ascertain whether all these cases represent the one disease. It would be preferable to confine the diagnostic label of CHL to those patients whose extensive and excessively long lanugo body hair is present at birth and affects all the usual hair-bearing areas of skin. The outcome is variable, with many authors reporting spontaneous loss of most of the abnormal hair in childhood while others refer to persistence and even progressive increase of hypertrichosis in adult life. In our patient, there has been a significant improvement during the first 6 months.

Developmental glaucoma has been associated with various dental and skeletal malformations: hypo- or adontia, facial anomalies such as hypertelorism, telecanthus, broad flat nose and maxillary hypoplasia. These congenital defects are thought to be due to abnormal neural crest cell-directed development.[6] However, the association between glaucoma and CHL has not been reported. The early identification and treatment of glaucoma is important as the results of primary surgery are good.[7] Following his successful surgery, this patient will need to be closely followed up for the rest of his life as he is at risk of developing a recurrence of raised intraocular pressure in the right eye and in the abnormal left eye.

Acknowledgments

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References

1 Virchow R. Die Russischen Haarmenschen. *Berlin Klin Wschr* 1873; 10: 337–9.
2 Beighton P. Congenital hypertrichosis lanuginosa. *Arch Dermatol* 1970; 101: 669–72.
3 Suskind R, Esterly NB. Congenital hypertrichosis universalis. *Birth Defects: Original article series* 1971; 7 (8): 103–6.
4 Freire-Maia N, Felizali J, de Figueiredo AC *et al.* Hypertrichosis lanuginosa in a mother and son. *Clin Genet* 1976; 10: 303–6.
5 Partridge JW. Congenital hypertrichosis lanuginosa: neonatal shaving *Arch Dis Child* 1987; 62: 623–5.
6 Shields MB. *Textbook of Glaucoma.* Baltimore: Williams and Wilkins, 1987.
7 McPherson SD Jr, Berry DP. Goniotomy versus external trabeculotomy for developmental glaucoma. *Am J Opthalmol* 1983; 95: 427–31.

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A case report of a patient with features of systemic lupus erythematosus and linear IgA disease

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Summary A case is reported of a 35-year-old woman with a 22-year history of systemic lupus erythematosus (SLE) who later developed a blistering eruption compatible with the diagnosis of linear IgA disease.

Since 1979 linear IgA disease has been differentiated as a distinct entity from that of dermatitis herpetiformis by the homogeneous linear deposition of IgA at the basement membrane zone (BMZ) of the epidermis and with a lack of an associated gluten-dependent enteropathy.[1–3]

Case report

A 35-year-old woman had from the age of 13 a history of intermittent arthralgia, fever and anaemia. She was diagnosed as having systemic lupus erythematosus and in April 1985 she was seen at the Mannheim Clinic with fever and having had a haemoptysis. A perimyocarditis and pericardial effusion with an alveolitis and bilateral pleural effusions were present, and 5 days after admission she developed an erythematous rash on the trunk and thighs with several blisters on the right thigh. Vesicles later appeared on the buccal mucous membrane and on the tongue. Oedema of both legs and purpura was associated with a vasculitis and a thrombocytopenia. Anti-DNA antibodies and antinuclear antibodies in a titre of 1 : 640 were present. The serological findings were compatible with a diagnosis of systemic lupus erythematosus (SLE).

The patient was initially treated with 100 mg prednisone daily and with an improvement of the symptoms the dose was reduced to 60 mg daily and she was then discharged from hospital. She remained well over the next 4 years until March 1989, when the dose of prednisone was reduced further to 2·5 mg daily. In mid-July 1989 she developed blisters in itchy areas of erythema on the abdomen and thighs. She later had an arthralgia with fever and diarrhoea and was admitted to hospital in Heidelberg. She was pyrexial and there were urticated areas of erythema and large tense blisters arranged in a herpetiform manner on her abdomen and left thigh (Fig. 1). There was no involvement of the mucous membranes.

Laboratory investigations

Haemoglobin was 11·7 g/dl, WBC $24{\cdot}9\times10^9$/l, with 50% neutrophils, 36% lymphocytes, 6% monocytes, 2% atypical lymphocytes. The platelet count was 451,000/ml and the ESR was 37 mm/h. The serum immunoglobulins were IgG 20·5 g/l, IgA 2·14 g/l and IgM 2·25 g/l. Echocardiography revealed a small pericardial effusion and there was also a small left-sided pleural diffusion.

Histopathology

A skin biopsy from the medial aspect of the thigh showed a subepidermal bulla, the lumen of which was filled with fibrinoid material and inflammatory cells that consisted mainly of lymphocytes and eosinophils (Fig. 2). There was a marked lymphohistiocytic infiltration of the upper dermis with numerous eosinophils and a perivascular-mixed infiltrate in the dermis.

Immunopathology

Four separate skin biopsies were examined by direct immunofluorescence (DIF) and the findings are shown in Table 1. In all four biopsies there was a homogeneous linear deposition of IgA at the dermo-epidermal junction zone (Fig. 3). This was most intense in the biopsy taken of a fresh lesion from non-sun-exposed skin and showed only subclass IgA1. In the lesional non-sun-exposed skin there was also an additional faint homogeneous deposi-

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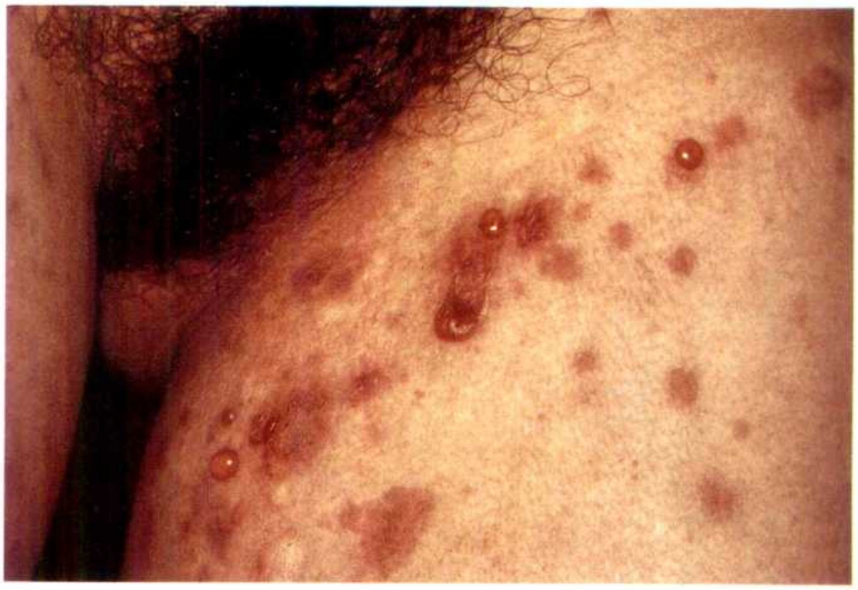

Figure 1. Vesiculobullous eruptions on the medial aspect of the thigh.

tion of IgM at the BMZ. This was confirmed using the more sensitive APAAP method.[4]

The BMZ showed deposits of C4d, C3b, C3d in an unusual pattern with involvement of the epidermal basal layer (Fig. 4). The capillaries in lesional skin from the papillary dermis showed deposits of C4d, C3d (Fig. 4), C5 and C9 together with focal deposits of fibrin in the upper dermis away from the BMZ.

The serum of the patient was screened for autoantibodies and the only significant finding was the presence of IgA autoantibodies to the BMZ using monkey oesophagus and in a titre of 1 : 5. The presence of circulating IgA antibodies directed to the BMZ was confirmed on cryostat sections of human skin.[5] A high titre of 1 : 20 was found. The bound IgA was localized in the split skin exclusively to the epidermal side (Fig. 5). An identical pattern was obtained with the serum from another patient with linear IgA disease. The HLA haplotypes of our patient were A23, A24, B35, B57, Cw4 and Cw7.

Progress

Our patient was treated with prednisone at a dosage of 50 mg daily for 1 week with some improvement of her

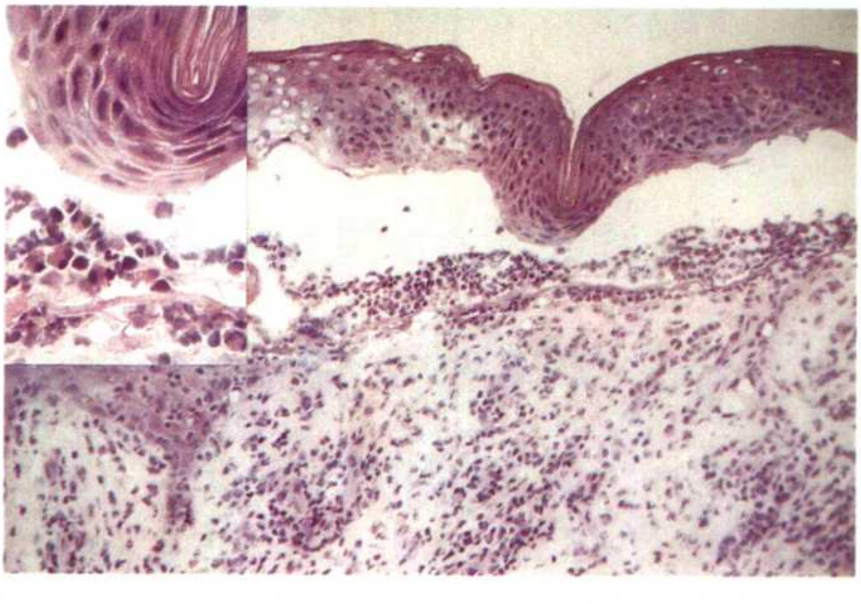

Figure 2. Cryostat section of subepidermal bulla in lesional sun-exposed skin showing a large leucocytic infiltration in the upper dermis consisting largely of eosinophilic granulocytes (Haematoxylin and eosin, × 100; insert × 400).

Table 1. DIF findings in four biopsies from different sites

Biopsy	Histology	IgG	IgM	IgA	C3d
LNEX	No bulla or cleft, multiple dense foci of infiltrating cells in upper dermis	–	(+)	+ BMZ	+ BMZ ICS of BK, PAPCA
LEX	Subepidermal blister, multiple dense perivascular foci of infiltrating cells in upper dermis	–	–	(+) l&c BMZ	+ pseudolinear BMZ, ICS of BK
NEX	Few perivascular foci of infiltrating cells, in upper dermis only	–	–	(+) l&d BMZ	++ l&c BMZ ICS of BK
NNEX	Few discrete perivascular cellular infiltrations, in upper dermis only	–	–	(+) l&d BMZ	–

LEX, lesional, sun-exposed; LNEX, lesional, non sun-exposed; NEX, normal, sun-exposed; NNEX, normal, non sun-exposed; ICS, intercellular space; BK, basal keratinocytes; PAPCA, papillary capillaries; l&c, linear and continuous; l&d, linear and discontinuous; linear, sharply defined deposits; pseudo linear, homogeneous, but ill-defined deposits along the DEJ.

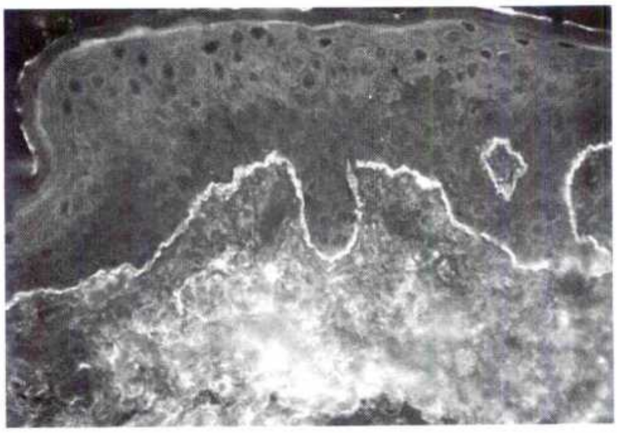

Figure 3. Homogeneous linear deposition of IgA at the BMZ in lesional, non-sun-exposed skin as revealed by DIF (× 250).

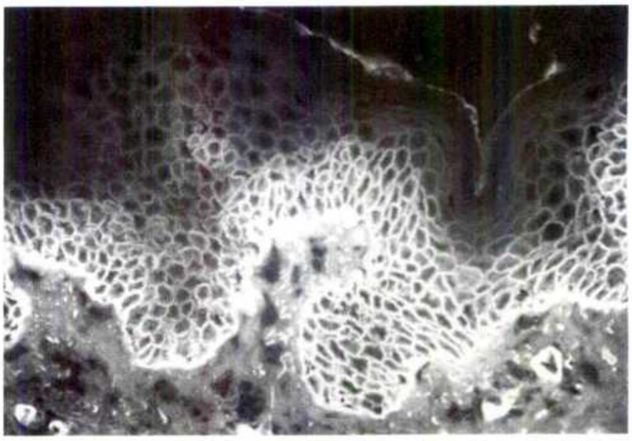

Figure 4. DIF of lesional non-sun-exposed skin, with C3d deposited at the BMZ and in the intercellular space of basal and suprabasal keratinocytes (× 250).

general symptoms and slight improvement of her skin eruptions. On stopping prednisone new blisters occurred and with the findings from DIF she was then treated with topical corticosteroids and 100 mg daily of dapsone. Within 1 week there was a marked improvement of her skin lesions and she was discharged from hospital. Several weeks later she had blisters on the buccal mucous membrane and stopped taking the dapsone and resumed treating herself with prednisone. Since then she has been lost to follow up.

Discussion

Our patient meets the criteria of the ARA[6] for SLE with an arthritis, polyserositis, coagulation disorder, thrombocytopenia, with ANF and antibodies against ds-DNA. The patient also had linear IgA disease with no evidence of a gluten enteropathy. The histology of the skin biopsies confirmed the diagnosis of linear IgA disease with a linear and homogeneous deposition of IgA at the dermo-epidermal junction, quite distinct from the IgA

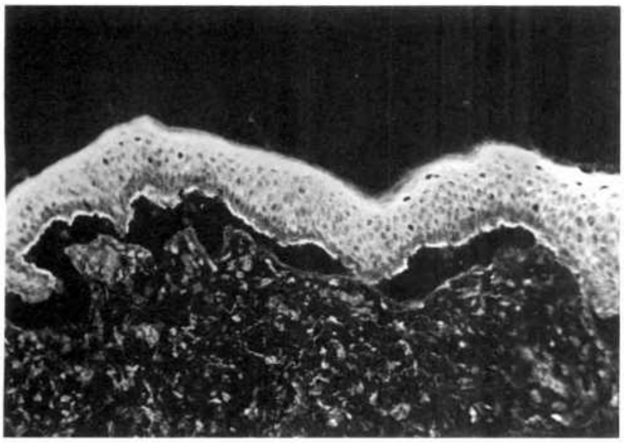

Figure 5. Human split skin incubated with the patient's serum diluted 1:10. IgA is localized to the epidermal side (× 400).

pattern occasionally seen in SLE. In addition there was IgM deposition as has been previously reported in linear IgA disease.[7] We found circulating antibodies that reacted to the epidermal portion of the basement membrane zone in split skin as has been reported in other cases of linear IgA disease.[8,9,10] These have recently been shown to react only with the epidermal basement zone of split skin and to recognize a 97-kDa antigen in dermal and epidermal extracts.[11] However, we obtained epidermal binding on split-skin substrate with our patient's serum, but could not find any binding to the linear IgA disease antigen. This may be due to a relatively low titre of the antibody.[11] The haplotype of our patient did not demonstrate HLA-B8 as found in 80–90% of patients with dermatitis herpetiformis and up to 30% of patients with linear IgA disease.[12]

Due to our patient's habit of taking prednisone for years without medical control or advice it was difficult to assess how effective dapsone was. It did appear to have some effect in reducing the blistering of her skin although it had little or no effect on the lesions of the buccal mucous membrane, which could be attributed to the concurrent SLE. Patients with bullous SLE may respond dramatically to dapsone or sulphapyridine, particularly those with circulating BMZ antibodies.[13–16] Those patients without antibodies divide into responders to dapsone[12,17–20] and those who do not.[16,17,21–23] Bullous eruptions in SLE are an uncommon manifestation of the disease rather than a co-existing independent bullous dermatosis. There have been four reports of the concurrence of SLE with a bullous dermatosis in the past two decades. In the light of recent findings a case of epidermolysis bullosa acquisita with a superimposed SLE where the clinical criteria for both SLE and EBA were met deserves attention[24]. In that case EBA preceded the onset of SLE by 5 years.

Two reports concerning SLE and concurrent DH have been published.[25,26] The only publication to date concerning concurrent SLE and linear IgA disease[27] was of a patient with SLE who later developed a rash compatible with dermatitis herpetiformis and the skin histology was typical for this disorder. However, DIF showed a strong linear deposition of IgA in the normal and involved skin, with faint immunoreactivity for IgG and IgM. This patient had no evidence of a gluten enteropathy or other circulating antibodies and did not have the HLA-B8 haplotype. The skin lesions in this patient did not respond well to corticosteroids but responded dramatically to dapsone.

This report is the second since 1983 of a patient with concurrent SLE and linear IgA disease. We were also able to demonstrate circulating BMZ antibodies in our patient.

Acknowledgments

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References

1 Chorzelski TP, Jablonska S, Beutner E H. Adult form of linear IgA bullous dermatosis. In: *Immunopathology of the Skin* (Beutner EH, Chorzelski TP, Bean SF, eds), 2nd edn. New York: John Wiley & Sons, 1979; 316.

2 Leonard JN, Haffenden GP, Ring NP *et al.* Linear IgA disease in adults. *Br J Dermatol* 1982; **107:** 301–6.

3 Smith SB, Harrist TJ, Murphy GF *et al.* Linear IgA bullous dermatosis v. dermatitis herpetiformis. *Arch Dermatol* 1984; **120:** 324–8.

4 Cordell JL, Falini B, Erber WN *et al.* Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 1984; **32:** 219–29.

5 Gammon WR, Briggaman RA, Inman AO *et al.* Differentiating anti-lamina lucida and anti-sublamina densa anti-BMZ antibodies by indirect immunofluorescence on 1·0 M sodium chloride-separated skin. *J Invest Dermatol* 1984; **82:** 139–44.

6 Tan EM, Cohen AS, Fries JF *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; **25:** 1271–7.

7 Leonard JN, Haffenden GP, Unsworth DJ *et al.* Evidence that the IgA in patients with linear IgA disease is qualitatively different from that of patients with dermatitis herpetiformis. *Br J Dermatol* 1984; **110:** 315–21.

8 Mobacken H, Kastrup W, Ljunghall K *et al.* Linear IgA dermatosis:

a study of ten adult patients. *Acta Dermatol Venereol (Stockh)* 1983, **63**: 123–8.

9 Pehamberger H, Konrad K, Holubar K. Circulating IgA anti-basement membrane antibodies in linear dermatitis herpetiformis (Duhring): immunofluorescence in linear distribution and immunoelectron microscopic studies. *J Invest Dermatol* 1977; **69**: 490–3.

10 Yaoita H, Katz SI. Circulating IgA antibasement membrane antibodies in dermatitis herpetiformis. *J Invest Dermatol* 1977, **69**: 558–61.

11 Zone JJ, Taylor TB, Kadunce DP, Meyer LJ. Identification of the cutaneous basement membrane zone antigen and isolation of antibody in linear immunoglobulin A bullous dermatosis. *J Clin Invest* 1990; **85**: 812–20.

12 Lawley TJ, Strober W, Yaoita H, Katz SI. Small intestinal biopsies and HLA types in dermatitis herpetiformis patients with granular and linear IgA skin deposits *J Invest Dermatol* 1980; **74**: 9–12.

13 Gammon WR, Woodley DT, Dole KC, Briggaman RA. Evidence that anti-basement membrane zone antibodies in bullous eruption of systemic lupus erythematosus recognize epidermolysis bullosa antigen. *J Invest Dermatol* 1985; **84**: 472–6

14 Barton DP, Fine J-D, Gammon WR, Sams WM. Bullous systemic lupus erythematosus: an unusual clinical course and detectable circulating autoantibodies to the epidermolysis bullosa acquisita antigen. *J Am Acad Dermatol* 1988; **15**: 369–73.

15 Kettler AH, Bean SF, Duffy JO, Gammon WR. Systemic lupus erythematosus presenting as a bullous eruption in a child. *Arch Dermatol* 1988; **124**: 1083–7.

16 Rappersberger K, Tschachler E, Tani M, Wolff K. Bullous disease in systemic lupus erythematosus. *J Am Acad Dermatol* 1989, **21**: 745–52.

17 Camisa C, Sharma HM. Vesiculobullous systemic lupus erythematosus. Report of two cases and a review of the literature. *J Am Acad Dermatol* 1983; **9**: 924–33.

18 Hall RP, Lawley TJ, Smith HR, Katz SI. Bullous eruption of systemic lupus erythematosus. Dramatic response to dapsone therapy. *Ann Int Med* 1982; **97**: 165–70.

19 Pedro SD, Dahl MV. Direct immunofluorescence of bullous systemic lupus erythematosus. *Arch Dermatol* 1973; **107**: 118–20.

20 Tani M, Shimizu R, Ban M *et al* Systemic lupus erythematosus with vesiculobullous lesions. Immunoelectron microscopic study. *Arch Dermatol* 1984; **120**: 1497–1501.

21 Jacoby RA, Abraham AA. Bullous dermatitis and systemic lupus erythematosus in a 15-year-old boy. *Arch Dermatol* 1979; **115**: 1094–7.

22 Olansky AJ, Briggaman RA, Gammon WR *et al.* Bullous systemic lupus erythematosus. *J Am Acad Dermatol* 1982; **7**: 511–20.

23 Penneys NS, Wiley HE. Herpetiform blisters in systemic lupus erythematosus. *Arch Dermatol* 1979; **115**: 1427–8.

24 Dotson AD, Raimer SS, Pursley TV, Tschen J. Systemic lupus erythematosus occurring in a patient with epidermolysis bullosa acquisita. *Arch Dermatol* 1981; **117**: 422–6.

25 Davies MG, Marks R, Waddington E. Simultaneous systemic lupus erythematosus and dermatitis herpetiformis. *Arch Dermatol* 1976; **112**: 1292–4.

26 Aronson AJ, Soltani K, Aronson IK, Ong RT. Systemic lupus erythematosus and dermatitis herpetiformis. Concurrence with Marfan's syndrome. *Arch Dermatol* 1979; **115**: 68–70.

27 Thaipisuttikul Y, Piamphongsant T, Suwanela N. Coexistence of linear IgA dermatitis herpetiformis and systemic lupus erythematosus. *J Dermatol* 1983; **10**: 161–6.

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Comment

Cosmetics and drugs. Is there a need for a third group: cosmeceutics?

Several skin conditions that were originally treated with cosmetics, such as male pattern baldness, dandruff and skin ageing wrinkles, are now considered to be treatable skin diseases. There is however a risk that pharmaceutically active products that have a greater risk of side-effects are being recommended for cosmetic use.

According to the Federal Food Drug and Cosmetic Act (FD&C) a cosmetic is defined as 'an article intended to be applied to the human body... for cleansing, beautifying, promoting attractiveness, or altering the appearance without affecting the body's structure or function.'[1] The definition as used by the EEC is similar, but the phrase 'without affecting the body's structure or function' is not included.[2] A drug is defined by the FD&C as 'an article intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease... (or) intended to affect the structure or any function of the body'.[1] From these definitions it can be concluded that the nature of the product, whether a drug or a cosmetic, is determined by the statements on the label about the intended use of the product. The manufacturer marketing the product makes the initial step in determining its nature and the licencing agency will have to determined whether the proposed status of the product is correct.

Both in the United States and in EEC countries there are strict regulations concerning cosmetic products which may not cause damage to health or contain harmful substances.[2] However, the FD&C Act does not require, but strongly urges, that the manufacturers or marketers of cosmetics should test their products for safety. A cosmetic company may use any raw material as a cosmetic ingredient on its own responsibility apart from colour additives and a few ingredients that are prohibited. In the United States the entire formula for cosmetic products has to be displayed, but in the EEC such precise labelling is not obligatory. At present there is a proposal for a sixth amendment of the 1976 Cosmetic Directive of the EEC and the aim of this is to produce a list of all products that can be used in cosmetics and which have been tested for their safety. In the past, unwanted local and systemic side-effects have led to the withdrawal of some cosmetics or their ingredients. An example was the epidemic of photoallergic reactions due to halogenated salicylanilides and related compounds in 1960 and 1961.[3] When these were prohibited there was a rapid decline in this photocontact dermatitis.[4] An example of systemic side-effects was the use of hexachlorophene-containing cosmetic products which were responsible for the death of 36 babies in France in 1972, and as a consequence hexachlorophene was restricted for prescription use only.[5]

When new cosmetic products with a marked pharmaceutical activity are being developed, the possibility of systemic side-effects has to be considered. This was seen with topical minoxidil used in the treatment of androgenetic alopecia. In a randomized double-blind placebo-controlled study with two parallel groups, no change of blood pressure was observed in the group treated with topical minoxidil, but the heart rate, the cardiac output and left ventricular mass increased in this group.[6] The topical form of minoxidil was approved by the FDA and is licenced for use in the treatment of androgenetic alopecia.[7] The topical application of *trans*-retinoic acid serves as another example where systemic side-effects and pharmaceutical activity have to be considered. This drug is advocated for the prevention and treatment of photoageing but as a retinoid is teratogenic.[8] There is increasing evidence about the efficacy of topical retinoin in the prevention and treatment of photoageing,[9,10] but to our knowledge there is no conclusive data to exclude a teratogenic effect from its topical application on human skin. Recently there was evidence for the percutaneous absorption of isotretinoin from photo-isomeration of topical tretinoin.[11] In many countries, the topical application form of *trans*-retinoic acid is regarded as, and marketed as, a cosmetic. When an effect on photoaged skin is shown, this product could be regarded as a drug and in that situation photoaged skin as a pathological condition. However, at present both minoxidil and retinoic acid are included on the restricted EEC list of cosmetic items numbers 373 and 374.

Sunscreens are somewhat different although theoretically unlimited use can suppress vitamin D_3 production.[12] Nevertheless, sunscreens are considered in most European countries to be cosmetics.

We suggest a separate group of products that are neither pure drugs nor cosmetics and which would be called 'cosmeceutics'. These may have little pharmaceutical activity and potential side-effects and would be prescribed for what would be considered to be traditional cosmetic indications. Both topical minoxidil and *trans*-retinoic acid could be examples of cosmeceutics.

In the U.S.A. most of these products such as anti-dandruff shampoos, anticaries toothpaste, sunscreens and antiperspirants are over-the-counter drugs (OTC).[13] In the EEC the situation is more complex, and the criteria for drug delivery being prescription only (PO) as against OTC varies from one member state to another. At present there is a directive being prepared to define the legal status for the supply of drugs for human use by PO as against OTC.

In conclusion, it is likely that in the field of cosmetics, new pharmacologically active compounds will be found and the distinction between drugs and cosmetics will become increasingly more blurred. We propose that products that are intended to be used for traditional cosmetic indications and which have little pharmaceutical activity and potential side-effects should be termed cosmeceutics. The efficacy of these products should be assessed in well defined and performed studies. The requirements for these studies as regards the assessment of side-effects should be as strict as those for the assessment of drugs.

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References

1 The Food, Drug and Cosmetic Act; 21 United States Code 301-92

2 Cosmetic Directive 76/768/EEC, article 1

3 Wilkinson DS Photodermatitis due to tetrachlorosalicylanilide. *Br J Dermatol* 1961; 73: 213–9.

4 Smith SZ, Epstein JH. Photocontact dermatitis to halogenated salicylanilides and related compounds. *Arch Dermatol* 1977; 113: 1372–4.

5 Editorial. Hexachlorophene today. *Lancet* 1982; 1: 87–8.

6 Leehen FHH, Smith DL, Unger WP. Topical minoxidil. cardiac effects in bald men. *Br J Clin Pharmac* 1988; 26: 481–5

7 Topical minoxidil approved by FDA *Clinical Pharmacy* 1988; 7: 858–62.

8 Sun M. Anti-acne drug poses dilemma for FDA. *Science* 1988; 240: 714–5.

9 Kligman AM, Grove GL, Hirose R, Leyden JJ. Topical tretinoin for photoaged skin. *J Am Acad Dermatol* 1986; 15: 836–59

10 Leyden JJ, Grove GL, Grove MJ *et al.* Treatment of photodamaged facial skin with topical tretinoin. *J Am Acad Dermatol* 1989; 21: 638–44

11 Lehman PA, Malany AM. Evidence for percutaneous absorption of isotretinoin from the photo-isomerization of topical tretinoin. *J Invest Dermatol* 1989; 93: 595–9

12 Matsuoka LY, Wortsman J, Hanifin N, Holick MF. Chronic sunscreen use decreases circulating concentrations of 25-hydroxy-vitamin D. *Arch Dermatol* 1988; 124: 1802–4.

13 Dandruff, seborrhoeic dermatitis and psoriasis drug products for over-the-counter human use. Tentative final monograph. *Federal Register* 1986; 51, no 146: 301–15.

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mycoses

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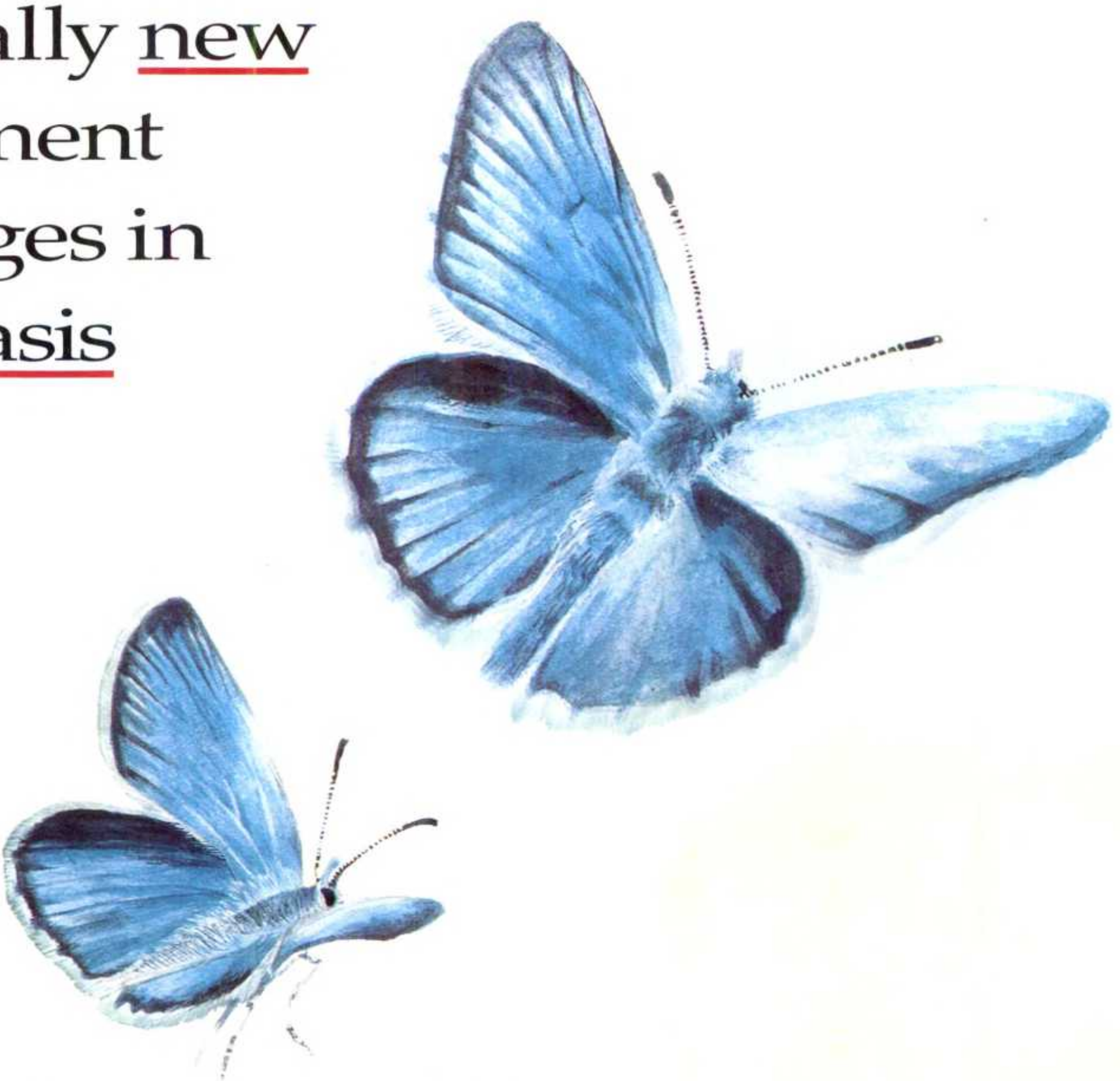

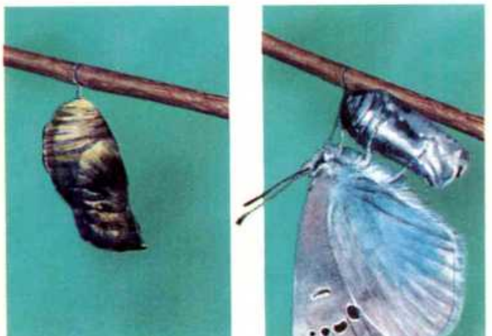

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Reduced therapeutic effect of warfarin caused by etretinate

Sir, Etretinate, an aromatic analogue of retinoic acid is an established therapy for psoriasis. It may also be indicated in a number of other skin conditions including cutaneous T-cell lymphoma, when it is often used in combination with photochemotherapy (PUVA).[1] We report a patient with T-cell lymphoma on warfarin who was started on etretinate and required a significant increase in coumarin dosage to be adequately anticoagulated. This interaction has not been previously described.

A 63-year-old man presented in December 1989 with dry mouth and eyes, polyarthropathy, fever, widespread lymphadenopathy, hepatosplenomegaly and a generalized erythematous papular rash. A skin biopsy showed a perivascular T-cell infiltrate and an axillary node biopsy showed evidence of a T-cell lymphoma.

He initially responded well to six courses of cyclophosphamide, adriamycin, vincristine and prednisolone. The rash, however, became nodular with severe pruritus and he was admitted and treated with prednisolone 25 mg daily and PUVA. He was started on tolbutamide because of hyperglycaemia (blood glucose 25 mmol/l). Shortly after admission he developed pleuritic chest pain and a ventilation-perfusion scan showed a mismatch consistent with pulmonary embolism. He was heparinized and warfarin was started. After 14 days he was adequately anticoagulated on 7 mg warfarin daily (Fig. 1). Three weeks after admission he was started on etretinate 40 mg daily which was followed by a significant reduction in the international normalised ratio (INR) to a non-therapeutic level. An increase in warfarin to 10 mg was necessary to resume adequate anticoagulation. Liver function tests were normal throughout this period.

To our knowledge, no interaction between etretinate and warfarin has been described. Hartmann *et al.*[2] studied the interaction of acitretin, a metabolite of etretinate, and phenprocoumon, a coumarin, in healthy volunteers. They concluded that acitretin did not significantly affect the anticoagulant action of phenprocoumon. In our patient an increase in dose of warfarin was necessary to reestablish an adequate INR after etretinate was started. Other potentially interacting drugs (tolbutamide, 8-methoxypsoralen, prednisolone, brompheniramine and cimetidine) were started soon after admission, 3 weeks before starting etretinate. Coproxamol (paracetamol 325 mg and dextropropoxyphene 32·5 mg) two tablets four times per day, was started 3 days after the etretinate. This drug, however, has been shown to potentiate the therapeutic effect of warfarin, probably due to competition between propoxyphene and warfarin for hepatic microsomal enzymes.[3]

The cause of this warfarin–etretinate interaction is not known. Studies in rats have shown some induction of hepatic mixed-function monoxygenase by etretinate[4] and it is possible this has caused an increased rate of metabolism of warfarin in this case. The INR should be closely monitored in patients on warfarin who are started on, or have a change in their dose of etretinate. With the increasing use of etretinate it is important to be aware of this potentially serious interaction with warfarin.

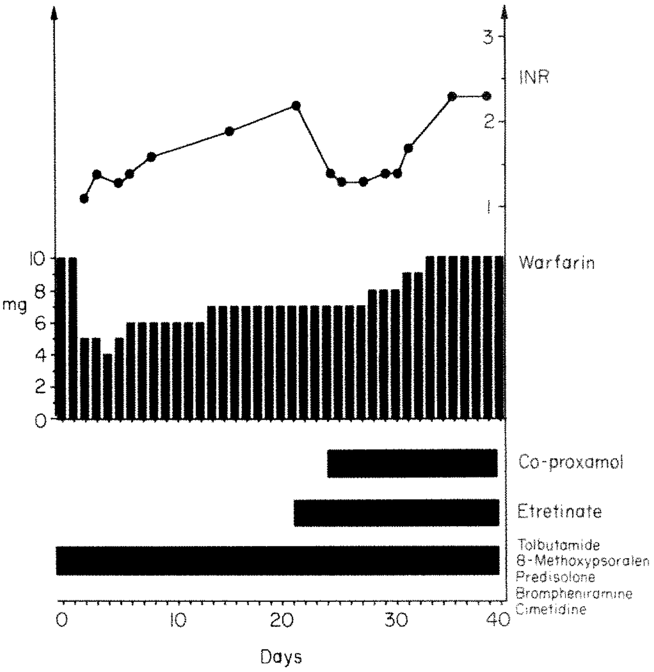

Figure 1. Drug treatment and INR during admission.

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References

1 Hunziker T, Zala L, Krebs A. Retinoid—orale photochemotherapie (Re-PUVA) als kombinationsbehandlung bei mycosis fungoides. *Dermatologica* 1983; **166**: 165–8.
2 Hartmann D, Mosberg H, Weber W. Lack of effect of acitretin on hypothrombinemic action of phenprocoumon in healthy volunteers. *Dermatologica* 1989; **178**: 33–6.
3 Smith R, Prudden D, Hawkes L. Propoxyphene and warfarin interaction. *Drug Intelligence and Clinical Pharmacy* 1984; **18**: 822.
4 Goerz G, Vizethum W, Kind R, Hornstein M. Influence of a new retinoid (Ro 10-9359) on the cytochrome P-450 system and other enzymes in rats' liver. *Dermatologica* 1978; **157** (Suppl. 1): 26–31.

Adonis 000709639100121W

Fatty liver following isotretinoin therapy

Sir, Although hepatotoxic reactions following treatment with etretinate are well documented[1–4] they are claimed not to occur with isotretinoin.[3] We report the development of extensive steatosis of the liver following a course of isotretinoin for the treatment of acne.

The patient presented at the age of 18 with a 6-year history of acne affecting his face, chest and back. It was unresponsive to oxytetracycline 1 g daily. He was otherwise well, was not taking any other medication and he drank one pint of cider per week. His hobby was weightlifting but he was not taking any performance-enhancing drugs other than desiccated liver tablets (Super Body Power—Healthilife Health Products). These contain desiccated ox liver and edible bone phosphate. He had no history of blood transfusions, contact with jaundice or travel abroad. He denied solvent abuse. There was no family history of liver disease.

On examination he had nodulocystic acne affecting his face, chest and back. Although weighing 95 kg he was 1·87 m tall and muscular. His fasting lipids and liver function tests were found to be normal (bilirubin, alanine aminotransferase, alkaline phosphatase) and he was started on a 4-month course of isotretinoin 40 mg daily (0·5 mg/kg). His acne resolved but his serum alanine aminotransferase (ALT) was found to be increased (134 u/l; normal range 7–46 u/l) at the end of the course. Bilirubin, alkaline phosphatase, gamma GT, hepatitis B antigen and autoantibody screen were all normal or negative. His ALT remained elevated for 6 months and a liver biopsy was carried out. This showed severe generalized steatosis of macrovesicular type with mild non-specific reactive inflammatory changes. A radioisotope scan showed slight hepatomegaly with even uptake. Other causes of hepatic steatosis were considered. He was not obese and a glucose tolerance test was normal. Alpha-1-antitrypsin levels were persistently at or below the lower limit of normal although his alpha-1-antitrypsin phenotype was heterozygote (PIMS) and typical diastase-resistant granules were not found in the liver biopsy. Over the subsequent 18 months, serum ALT remained elevated at 88–101 u/l.

Isotretinoin has been described as being devoid of significant hepatotoxicity[3] and we could find no published reports of such an effect. Only mild elevations of liver enzymes in 10% of patients have been reported with isotretinoin.[5,6] In the latter study the mean daily dose was 109 mg and mean duration of treatment was 150 days. The present case had a daily dose of less than half of this (40 mg) and duration of treatment was 112 days. However, one patient (personal communication, Roche Products Limited) developed abnormal liver function tests following a 5-month course of an unknown dosage of isotretinoin. Liver function returned to normal when the drug was stopped but became abnormal again after a further 3 months on the drug. Liver function failed to return to normal 18 months after stopping the drug and a liver biopsy revealed a mild chronic hepatitis with some fat.

Our patient's liver function has failed to improve over the 20 months since the drug was stopped. The low dose of isotretinoin suggests an idiosyncratic rather than toxic reaction. In view of this possibility, the observation of a phenotype associated with low-normal levels of α-1-antitrypsin is of interest. It may be postulated that this could render an individual more susceptible to such a reaction and could be related to the persistance of the hepatic abnormality after the drug was withdrawn. It should be noted that this phenotype is not rare, occurring in 9% of the U.K. population.[7] Unlike the PIMZ phenotype, it has not been associated with liver disease.

In view of the temporal relationship between the onset of the course of isotretinoin and the appearance of abnormal liver function, it is possible that isotretinoin in association with low-normal levels of α-1-antitrypsin may have been responsible. This observation suggests that monitoring of serum ALT concentrations should be undertaken during isotretinoin therapy.

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References

1 Weiss VC, West DP, Ackerman R, Robinson L. Hepatotoxic reactions in a patient treated with etretinate. *Arch Dermatol* 1984; **120**: 104–6.

2 Thune P, Mork NJ. A case of centrolobular toxic necrosis of the liver due to aromatic retinoid Tigason (Ro 10-9359). *Dermatologica* 1980, **160**: 405–8.

3 Vahlquist A, Loof L, Nordlinder H *et al.* Differential hepatotoxicity of two oral retinoids (etretinate and isotretinoin) in a patient with palmoplantar psoriasis. *Acta Derm Venereol (Stockh)* 1985; **65**: 359–62

4 van Voorst Vader PC, Houthoff HJ, Eggink HF, Gips CH. Etretinate (Tigason) hepatitis in two patients. *Dermatologica* 1984; **168**: 41–46.

5 Dicken CH. Retinoids: A review. *J Am Acad Dermatol* 1984; **11**: 541–52

6 Winhorst DB, Nigra T. General clinical toxicology of oral retinoids. *J Am Acad Dermatol* 1982; **6**: 675–82.

7 Kolshekev N. Techniques for the study of alpha-1-antitrypsin variants. *Association of Clinical Pathologists Broadsheet* 1985; **113**: 1–11.

ADONIS 000709639100122B

Pathogenesis of *in-vivo* antinuclear antibodies of the skin

SIR, Deposits of IgG can be observed in the nuclei of epidermal cells in skin biopsies taken from various systemic connective tissue disorders (SCTD). As passive diffusion of circulating antinuclear antibodies into living cells is unlikely, an artefact has been suspected[1] and this phenomenon has concerned many investigators.[2–4] Recent studies have given some insight into the pathophysiological mechanisms that may be involved.[5,6]

In a retrospective study of biopsies of skin from 244 patients with various SCTD, we found the presence of *in-vivo* antinuclear antibodies was significantly associated with antibodies in the serum against the non-histone nucleoproteins (nHNPs; McNemar's test, $P<0{\cdot}05$), but not with anti-dsDNA antibodies.[5] Because both anti-dsDNA and anti-nHNP antibodies can bind to epidermal cell nuclei in sections of human skin, such an association cannot be explained if the *in-vivo* ANA is caused by passive diffusion during the incubation of the sections. A special type of *in-vivo* deposition of IgG in both the nucleus and cytoplasm was first observed in the skin of patients with primary Sjögren's syndrome and was statistically associated with serum antibodies against SSA/Ro and SSB/La (Fisher's exact test, $P<0{\cdot}05$). This pattern was also later seen in patients with lupus erythematosus and who had monospecific antibodies against SSA/Ro.[6]

These findings indicate that *in-vivo* antinuclear antibodies of the skin are not disease-specific but an nHNP antibody-specific phenomenon, which probably represents the localization of the respective antigens in epidermal cells. It was observed using cultures and suspensions of human keratinocytes that anti-nHNP antibodies, but not anti-dsDNA antibodies, are bound to the outer membrane of the cells and these findings agree with those observed in the skin biopsies.[7-9] We suggest that antibodies against nHNP bind *in vivo* to the outer membrane of keratinocytes, and then are actively incorporated into the cell with their respective antigens.

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References

1 Prystowsky SD, Tuffanelli DL. Speckled (particulate) epidermal nuclear IgG deposition in normal skin. *Arch Dermatol* 1978; **114:** 705–10.

2 Iwatsuki K, Tagami H, Imaizumi S *et al.* The speckled epidermal nuclear immunofluorescence of mixed connective tissue disease seems to develop as an *in vitro* phenomenon. *Br J Dermatol* 1982; **107:** 653–7.

3 Chen Z-Y, Dobson RL, Ainsworth SK *et al.* Epidermal nuclear immunofluorescence: serological correlations supporting an *in vivo* reaction. *Br J Dermatol* 1985; **112:** 15–22.

4 Parodi A, Nunzi E, Rebora A. The speckled immunofluorescence of epidermal nuclei as an *in vivo* phenomenon. *Br J Dermatol* 1985; **112:** 555–8.

5 Velthuis PJ, Kater L, van der Tweel I *et al. In-vivo* ANA of the skin: its diagnostic significance and association with selective antinuclear antibodies. *Ann Rheum Dis* 1990; **49:** 163–7.

6 Velthuis PJ, Hené RJ, Nieboer C, Kater L. A prospective immunofluorescence study of immune deposits in the skin of primary Sjögren's syndrome. *Acta Derm Venereol (Stockh)* 1989; **69:** 487–92.

7 LeFeber WP, Norris DA, Ryan SR *et al.* Ultraviolet light induces binding of antibodies to selected nuclear antigens on cultured human keratinocytes. *J Clin Invest* 1984; **74:** 1545–51.

8 Furukawa F, Kashihara-Sawami M, Lyous MB, Norris DA. Binding of antibodies to the extractable nuclear antigens SSA/Ro and SSB/La is induced on the surface of human keratinocytes by ultraviolet light (UVL): implications for the pathogenesis of photosensitive cutaneous lupus. *J Invest Dermatol* 1990; **94:** 77–85.

9 Velthuis PJ, Wildschut EG, Baart de la Faille H *et al.* Binding of antibodies to non-histone nucleoproteins on keratinocytes in suspension. *Arch Dermatol Res* 1990; **282:** 159–63.

ADONIS 0007096391001230

Kerion masquerading as erosive pustular dermatosis of the scalp

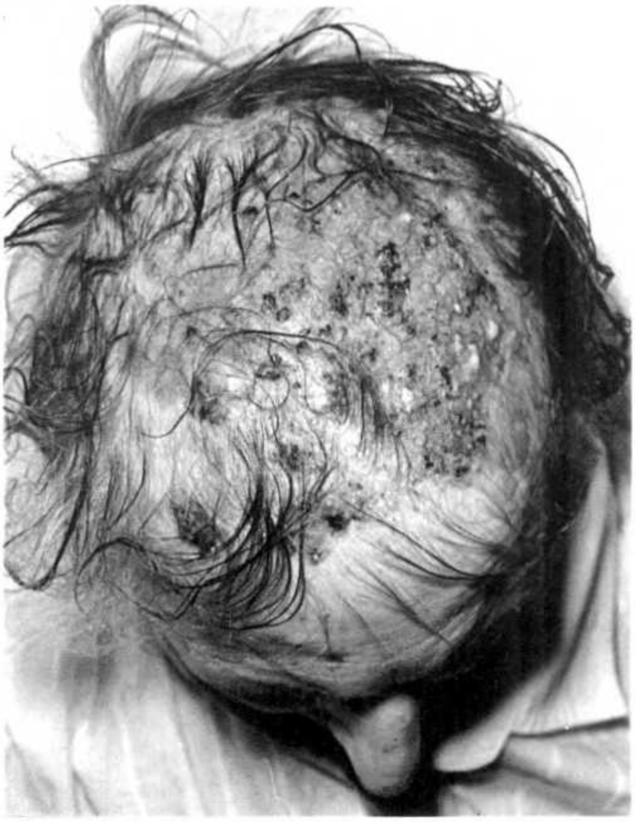

Figure 1. Appearance of the scalp eruption at presentation showing large pustules with scarring alopecia.

SIR, Erosive pustular dermatosis of the scalp as described by Pye *et al.*[1] lacks pathognomonic clinical or pathological features and the diagnosis rests on the exclusion of other dermatoses.[2] We report a recent case which illustrates the need for rigour in the exclusion of a dermatophyte infection before the diagnosis is made of erosive pustular dermatosis of the scalp.

A 76-year-old woman was referred with a 6-month history of a painful scalp eruption with extensive plaques of erythema and crusting as well as eroded areas with pustule formation (Fig. 1). She suffered from longstanding rheumatoid arthritis and was taking prednisolone 5 mg daily. She was also on cimetidine 200 mg b.d. for a duodenal ulcer that she had 4 years earlier. The clinical appearances were suspicious of a chronic fungal infection of the scalp, but Wood's light examination was negative and repeated examinations of potassium hydroxide preparations of scrapings from the scaly areas and of hairs were negative for fungal mycelia and spores. *Staphylococus aureus* was isolated from a scalp swab and she was treated with flucoxacillin 250 mg q.d.s. and mupirocin ointment with some improvement after 2 weeks. A scalp biopsy showed features of an erosive pustular dermatosis with superinfection by bacteria, but cultures for fungus were negative on two occasions.

Over the next 3 months, the scalp condition deteriorated with extension of the scarring alopecia. A further specimen of scalp crust, however, revealed on culture a growth of *Microsporum canis* and she was commenced on treatment with griseofulvin 500 mg b.d. Within 6 weeks there was dramatic improvement with complete clearing of the eroded and pustular lesions although she had an extensive residual scarring alopecia.

Mycological proof of scalp ringworm infection is not always easy to obtain and in one reported series, 30% of cases were culture negative.[3] The difficulty in isolating the pathogenetic fungus in this case leads us to suggest that erosive pustular dermatosis of scalp should not be diagnosed without a trial of griseofulvin which continues to be the agent of choice for treatment of kerion.[4]

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References

1 Pye RJ, Peachey RDG, Burton JL. Erosive pustular dermatosis of the scalp *Br J Dermatol* 1979; 100: 559–66.
2 Burton JL, Peachey RDG, Pye RJ. Erosive pustular dermatosis of the scalp—a definition. *Br J Dermatol* 1988; 119: 411.
3 Birt AR, Wilt JC Mycology, bacteriology and histopathology of suppurative ringworm. *Arch Dermatol* 1954; 69: 441–8.
4 Stephens CJM, Hay RJ, Black MM. Fungal kerion-total scalp involvement due to *Microsporum canis* infection. *Clin Exp Dermatol* 1990; 14: 442–444.

ADONIS 007096391001243

Venous ulceration and free radicals

Sir, The cause of venous ulceration remains unproven. Skin hypoxia resulting from pericapillary fibrin cuffs has been the favoured theory since its proposal by Browse and Burnand in 1982.[1] Dissenters point out that although fibrin cuffs exist, the evidence that they act as a diffusion barrier to the movement of oxygen is tenuous, resting as it does largely on the vagaries of transcutaneous oxygen measurement. They point to the fact that white cells are sequestrated in the leg when venous pressure is raised[2] and that the number of capillary loops visible on capillary microscopy of the gaiter region falls in venous patients when the limb is dependent.[3] These findings suggest that the capillaries may become occluded with leucocytes, which are activated as a result and cause enzymatic and free-radical damage to surrounding tissues. The two theories are not, of course, mutually incompatible; developments in our knowledge of free-radical chemistry suggest a third mechanism which may also be at work in venous disease.

The hydroxyl radical (OH·) is the most reactive free radical known. One of its principal sources in living systems is from iron, via the Fenton reaction:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH^- .$$

In health, most iron in the body is protein-bound and unavailable to take part in this reaction. In venous hypertension, there is emigration of erythrocytes into the interstitium through widened inter-endothelial pores and this has been demonstrated by electron microscopy.[4] Haemoglobin from lysed red cells is capable of releasing its iron in the presence of hydrogen peroxide,[5] which is formed when white cells are activated, and is capable of crossing cell membranes easily. Thus the following chain of events can be postulated: raised venous pressure leads to red cell extravasation. Haemoglobin from degraded erythrocytes releases iron which acts as a substrate for the Fenton reaction: H_2O_2 is supplied from white cells which have been activated by contact with endothelium. The two combine to generate OH· radicals. These cause local lipid peroxidation, leading to an inflammatory response. The attracted white cells generate further free-radical formation as a result of degranulation; this causes more lipid peroxidation, an increasing neutrophil response and a self-perpetuating cycle of tissue damage.

Such an hypothesis is complimetary to the activated white cell theory of venous ulcer pathogenesis, as it provides a mechanism by which the inflammatory response may be initiated. We are currently evaluating free-radical activity in venous patients; if fruitful, it will allow the possibility of scavenging agents being used therapeutically.

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References

1 Browse NL, Burnand KG. The cause of venous ulceration. *Lancet* 1982; ii: 243–5.
2 Moyses C, Cederholm-Williams SA, Michel CC. Haemoconcentration and accumulation of white cells in the feet during venous stasis. *Int J Microcirc Clin Exp* 1987; 5: 311–20.
3 Scott HJ, McMullin GM, Coleridge Smith PD, Scurr JH. Venous ulceration: the role of the white cell. *Phlebology* 1989; 4: 153–9.
4 Wenner A, Leu HJ, Spycher M, Brunner U. Ultrastructural changes of capillaries in chronic venous insufficiency. *Expl Cell Biol* 1980; 48: 1–14.
5 Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine* 2nd edn. Oxford: Clarendon Press, 1989.

ADONIS 00709639100125G

Treatment of Merkel cell tumour with interferon-α-2b

Sir, Interferons have been used in the treatment of viral papillomas, HIV-associated Kaposi's sarcoma, inflammatory dermatoses and cutaneous tumours.[1] We report a case of a Merkel cell tumour that was treated with interferon-α-2b (IFN).

An 85-year-old man had a subcutaneous mass in his right leg excised in March 1987 and the histology and immunohistochemistry were typical for that of a Merkel cell tumour. Postoperatively he received radiotherapy, but the tumour recurred and in December 1987 a further excision was performed. The patient later returned in March 1988 with a recurrent mass that measured 3 × 3 cm and following excision, he had a course of superficial radiotherapy. There was a further recurrence along the excision margin in May 1988. He was then treated with IFN (Intron A) receiving 3×10^6 U subcutaneously three times a week. The tumour became softer after 1 month and disappeared after 3 months. Subsequently there has been no recurrence with 1 year of follow up.

Merkel cell tumour is a rare but aggressive form of skin cancer and there is a high rate of recurrence of 35–40% within a year of diagnosis.[2,3] Locally recurrent lesions have been treated with variable success with further excision, amputation, radiation and chemotherapy.[2,3] The advanced age and the poor health of many of the patients preclude the routine use of cytotoxic drug therapy. This case report suggests that IFN may produce a relatively long remission in the treatment of Merkel cell tumour.

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References

1 Satler R, Mayer da Silva A, Bratzke B *et al.* Interferons in dermatology. *J Am Acad Dermatol* 1989; **20**: 650–6.
2 Raaf JH, Urmacher C, Knapper WK *et al.* Trabecular carcinoma of the skin, treatment of primary, recurrent and metastatic disease. *Cancer* 1986; **57**: 178–82.
3 Wynne CJ, Kearsley JH. Merkel cell tumour, a chemo-sensitive skin cancer. *Cancer* 1988; **62**: 28–31.

adonis 000709639100126T

Alopecia areata and topical sensitizers: allergic response is necessary, but irritation is not

Sir, We read with interest the letter concerning alopecia areata treated with diphencyprone (DPC)[1] and agree that the elicitation of an allergic reaction after the application of DPC is an integral part of successful treatment. However, confusion can arise when a distinction is not clearly made between irritation and an allergic response. Good therapeutic results are often obtained in the treatment of alopecia areata when low concentrations of an allergen are used and without symptoms and signs of irritation.[2] Treatment with irritants has proved unsuccessful.[3–5] With the 15 patients with alopecia areata who did not respond to DPC[1] the lack of success could be related to the short duration of therapy. A course of therapy lasting 20 weeks is too short for patients treated with topical sensitizers because the response can occur after 10 months[6] or even longer.[7,8] This is even more likely in those patients treated with lower concentrations of the allergen, as we have reported.[2]

Thus the distinction between immunological stimulation and irritation is of importance, the latter being considered non-essential for the induction of hair regrowth. We believe that topical sensitizers can be used successfully in the treatment of patients with alopecia areata without significant symptoms.[9,10]

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References

1 MacDonald Hull S, Cunliffe WJ, Norris JF. Alopecia areata treated with diphencyprone: is an allergic response necessary? *Br J Dermatol* 1990; **122**: 716–7.
2 Orecchia G, Rabbiosi G. Squaric acid dibutylester in alopecia areata: is discomfort really necessary? *J Am Acad Dermatol* 1987; **16**: 876.
3 Buchner U, Echternacht-Happle K, Happle R. Irritant dermatitis versus allergic contact dermatitis for the treatment of alopecia areata. *Arch Dermatol Res* 1979; **264**: 123.
4 Swanson NA, Mitchell AJ, Leahy MS *et al.* Topical treatment of alopecia areata. Contact allergy versus primary irritant therapy. *Arch Dermatol* 1981; **117**: 384–7.
5 Ashworth J, Tuyp E, MacKie RM. Allergic and irritant contact dermatitis compared in the treatment of alopecia totalis and universalis. A comparison of the value of topical diphencyprone and tretinoin gel. *Br J Dermatol* 1989; **120**: 397–401.
6 Happle R, Hansen BM, Wiesner-Menzel L. Diphencyprone in the treatment of alopecia areata. *Acta Derm Venereol (Stockh)* 1983; **63**: 49–52.
7 Orecchia G, Rabbiosi G. Treatment of alopecia areata with diphencyprone. *Dermatologica* 1985; **171**: 193–6.
8 Orecchia G, Douville H. A three-year treatment of alopecia areata with squaric acid dibutylester. *Ann It Derm Clin Sper* 1988; **42**: 179–85.
9 Barth JH, Darley CR, Gibson JR. Squaric acid esters in the treatment of alopecia areata. *J Am Acad Dermatol* 1986; **14**: 846.
10 Wilkin JK Squaric acid esters in the treatment of alopecia areata. *J Am Acad Dermatol* 1986; **14**: 846.

adonis 0007096391001278

Hypnotherapy for alopecia areata

Sir, There are anecdotal reports of hypnotherapy helping alopecia areata. We designed a trial to assess the effect of hypnotherapy on patients with extensive alopecia areata.

Table 1. Effect of hypnotherapy on hair growth

Patient	Age (years)	Sex	Alopecia	Psychological factors	Ease of induction of hypnosis	Change in alopecia
1	54	F	Total, non-scarring	A2 R3 C2	1	No change
2	45	M	Subtotal, non-scarring (patchy fine hair on scalp)	A1 R3 C1	2	Slight increase in fine hair on scalp
3	64	F	Total, mild scarring	A2 R1 C1	3	Development of stubbly eyelashes and eyebrows
4	33	F	Total, mild scarring	A3 R3 C3	2	Patchy fine hair on axillae and cheeks
5	19	M	Partial, non-scarring (approx. 90% of scalp)	A2 R2 C2	3	Significant increase of hair growth on scalp

A, anxiety; R, ability to relax; C, ability to concentrate; graded from 0 (no undue problems) to 4 (very significant problems).

Twelve patients (four males and eight females, age range 19–64 years, mean 43 years) were initially included in the study and all had total or subtotal alopecia for more than 5 years. Their previous therapy included topical and intralesional steroids, sensitization with DNCB or diphencyprone or treatment with topical cyclosporin. These treatments were ineffective and produced no significant hair growth. Because of the persistence of their alopecia, all the patients had a biopsy and four were found to have mild dermal scarring.

Of the patients asked to participate in this trial of hypnotherapy, only five (Table 1) completed the study. In an introductory session each patient was assessed psychologically and the degree of alopecia estimated. The patients then attended for 10–12 sessions of hypnotherapy, each session lasting about 45 min. The patients initially attended weekly and afterwards at 2–3 week intervals. Each patient was issued with a therapy tape that they continued to use on a regular basis. A variety of hypnosis techniques were used with each patient, including direct and indirect suggestion together with ego strengthening. The duration of the course of hypnotherapy was 3 months.

Psychological assessment prior to therapy indicated that none of the patients had chronic depression, although most of them had problems with anxiety. All the patients who completed the course of hypnotherapy reported a feeling of general well-being. The effect of the hypnotherapy on hair growth is summarized in Table 1. One patient had no change as regards her alopecia and three had minimal regrowth of hair. Only one patient with partial and non-scarring alopecia had a significant increase in hair growth on the scalp. From our limited study it appears unlikely that hypnotherapy can encourage useful regrowth of hair in alopecia areata, although it can improve psychological parameters in these patients.

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ADONIS 000709639100128L

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Book Reviews

Dermatology. 1st edition. Edited by M.Orkin, H.Maibach and M.V.Dahl (1991). Norwalk, Connecticut: Appleton & Lange. Pp. 695. ISBN 0-8385-1288-7.

The editors of this book want to serve the needs of too wide a range of medical and paramedical personnel including medical students and practising dermatologists. They have tried to find a niche between the smaller introductory texts and the larger specialist reference books. Their brief was perhaps too ambitious and not made easier by trying to make 65 contributors adopt a uniform format and style.

The book's 700 pages are divided into 55 chapters in 10 sections. Inevitably, in a multiauthor publication, the depth of approach varies. The main cause of this is the strict 'Lange format' in which a list of 'essentials of diagnosis' is followed by a general description and clinical findings of the disease and by sections on prevention and treatment. I found this irritating because, for example, atopic dermatitis and psoriasis are covered in no more words than the ectodermal dysplasias. As a result 90% of the workload of the average dermatologist takes up 7% of the pages. Another problem with this style is that its uniformity, coupled with the limited number of poor black and white illustrations, and the many hard-to-follow tables, becomes monotonous.

There are, of course, many good contributions. I particularly enjoyed the novel (for introductory texts) chapters on the care of the normal skin and on percutaneous absorption. The section on cutaneous infections, including AIDS, is also excellent.

Many of the deficiencies of the book are balanced by its low price which is astonishing for its size. I am not sure that it will appeal to all readers mentioned by the editors but I think this is a reasonable book for first year dermatology residents and new career registrars to buy. The book is far too long for medical students, and too rarity conscious for primary care physicians. Both groups would do better with the punchier texts, with many good colour photographs, which are now available at much the same price.

D.Kemmett

Practice of Skin Surgery. P.N.Behl (1990). New Dehli: Skin Institute and School of Dermatology. Pp. 140. Price 200 Rupees.

For those who look upon dermatological surgery as an extension of cosmetic surgery, fit only for the self-indulged West, the publication of this thin volume on the surgical aspects of dermatology designed for third world dermatologists must seem incongruous. In 24 chapters the author briefly covers topics ranging from basic surgical techniques and equipment through to dermabrasion, liposuction and vein surgery. There are chapters on vitiligo surgery and surgery in leprosy, which naturally have a special significance for the audience that the book is aimed at. Professor Behl, who has also written a host of other texts on clinical dermatological subjects, has tried hard to encourage careful surgical practice in the third world in this first edition. However, the book is not such an improvement on the many European and American dermatological surgery text books that I would recommend it over them. The literary style can be distracting, the diagrams amateurish and the clinical photographs are of poor quality. These faults could be remedied by a slicker publisher but he could not improve on the bindings—I am sure this book will not come apart in my hands after 2 years like so many Western offerings.

C.Lawrence

Clinical Dermatology. By J.A.A.Hunter, J.A.Savin and M.V.Dahl (1989). Oxford: Blackwell Scientific Publications. Pp. 272. Price £17.50

There are quite a number of good textbooks of clinical dermatology that are now available that are aimed for medical students and what the authors of this book consider to be 'the growing number of visually illiterate doctors who quail before the skin'. This is an excellent textbook, being well laid out with a clear and concise text that covers in some detail all the common conditions. There are many high quality colour photographs and in most of them the reproduction of the colour is good. However, a few of them could be improved upon, such as the one showing a case of dysplastic naevus syndrome which could show more detail of the lesions and was there a halo naevus among the moles? There was also the photograph of 'one or two shy adult pubic lice' (p. 168) which are invisible to my eye. Also, perhaps because it is referred to in the text (p. 155), it would have been appropriate to have a picture showing the bruise-like lesions of Kaposi's sarcoma occurring in patients with AIDS. In addition to the clinical photographs there were a number of line diagrams showing the distribution patterns of various dermatoses and many useful tables giving lists of causes for various conditions. All the common skin disorders were covered as well as many of the more rare conditions. An excellent feature in this textbook was the learning points interspersed and boxed in the text. The sections on the structure and function of the skin as well as that on therapy were in my opinion too much for medical students and perhaps not enough for postgraduates. However, as an optimist I hope that my medical students will buy and read this textbook which I am sure will become a very popular one.

S.S.Bleehen

British Journal of Dermatology (1991) 124, 512

News and Notices

2nd International Conference on the Biological Effects of UVA Radiation, 27–28 June 1991, San Antonio, Texas

An international conference on The Biological Effects of UVA Radiation will be held on 27–28 June 1991 in San Antonio, Texas, immediately following the 19th Annual Meeting of the American Society for Photobiology. The conference is sponsored by the American Society for Photobiology and will be chaired by Dr F.Urbach. The subjects discussed will include sources of UVA radiation, biological effects of UVA, action spectra for UVA effects, photocarcinogenesis by UVA, effects on immune function and protective agents against UVA.

For further details and information and registration forms contact: The Secretariat, American Society for Photobiology, 8000 Westpark Drive, McLean, VA 22102, U.S.A. Fax no.: 703-790-9063.

The Skin Disease Research Fund

Applications are invited for research grants from The Skin Disease Research Fund and the deadline for submission is 2 September 1991. Only the Fund's own forms will be accepted and these can be obtained from: the Secretary, The Skin Disease Research Fund, 3 St Andrew's Place, London NW1 4LB. Telephone 071-935-8576.

British Society for Investigative Dermatology, Leicester, 26–27 September 1991

The next annual meeting of the British Society for Investigative Dermatology will be held in Leicester on 26–27 September 1991. Abstracts are invited for consideration by the Selection Committee. Official abstract forms and further information from: Mrs R.Barton, The British Association of Dermatologists, 3 St Andrew's Place, Regent's Park, London NW1 4LB. The closing date for receipt of completed abstract forms is 5 June 1991.

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The ***Journal of Medical and Veterinary Mycology*** is a bi-monthly international journal publishing original articles that describe innovative research work on all aspects of invasive fungal disease in man and animals. These include mycological, biochemical and molecular studies of the causal fungi; aspects of pathogenesis, immunology, and epidemiology; case reports of unusual infections; clinical and laboratory approaches to diagnosis; antifungal therapy and prophylaxis; and the mode of action, pharmacokinetics and assessment of new antifungal agents in animals and humans. The prime aim of the journal is to present the best work in its field from throughout the world, providing a comprehensive reference base both for medical mycologists and for clinicians, infectious disease physicians and microbiologists who encounter fungal problems in their routine clinical and/or laboratory practice.

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Journal of Medical and Veterinary Mycology is published by Blackwell Scientific Publications, bimonthly from February 1991. A subscription to Volume 29, 1991 costs: £135.00 (UK), US$265.00 (USA & Canada) or £145.00 (overseas) post free.

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The journal is a forum for new information about the direct and distant effects of electromagnetic radiation (ultraviolet, visible, and infrared) mediated through skin. The divisions of the editorial board reflect areas of specific interest: aging, carcinogenesis, immunology, instrumentation and optics, photosensitivity, pigmentation, and therapy. *Photodermatology, Photoimmunology & Photomedicine* includes original articles, reviews, communications, and editorials. Original articles may include the investigation of experimental or pathological processes in humans or animals *in vivo* or the investigation of radiation effects in cells or tissues *in vitro*. Methodology need have no limitation: rather, it should be appropriate to the question addressed. Thus, all contemporary physiologic, microscopic, and biochemical methodologies are welcomed.
Review articles, including new trends, are ordinarily solicited by the editors, although we invite suggestions from all readers. Communications are intended for the reporting of a special observation, either clinical or experimental, to address an hypothesis, to make a single point, or to pose a question.

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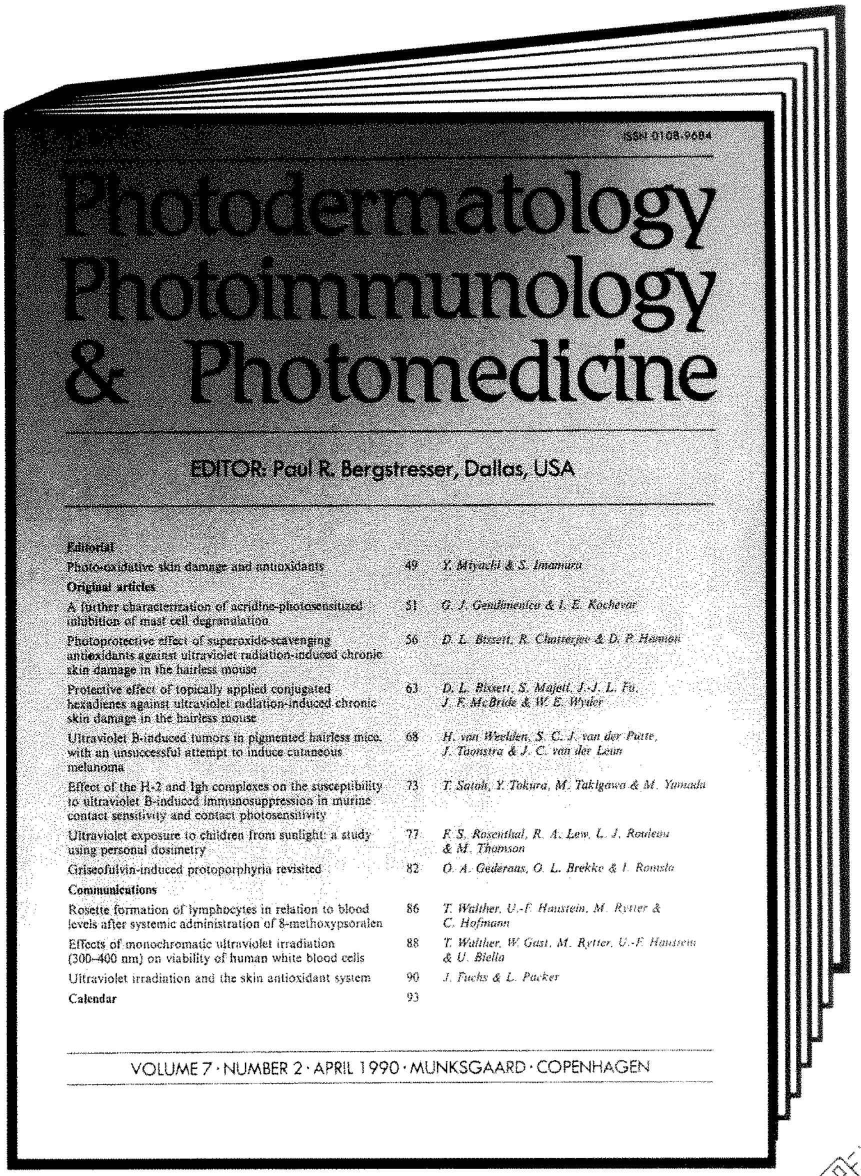

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JOURNAL OF THE BRITISH SOCIETY FOR ALLERGY AND CLINICAL IMMUNOLOGY
Edited by **A.B. Kay** and **S.T. Holgate**

Clinical Allergy has been known as ***Clinical and Experimental Allergy*** since 1st January 1989 and is the official journal of the British Society for Allergy and Clinical Immunology. The success of *Clinical Allergy* resulted largely from the ability to supply practising allergists with information based on clinical science experimentation, yet at the same time the editors maintained the interests of basic scientists. This tradition has been continued, and also improved upon. Review articles are included together with editorials, letters to the editor and book reviews. Some new features that have been incorporated include *current abstracts, allergy practice forum*, and *news and commentary*. Papers on drug trials that shed light on pathogenetic mechanisms or present the results of entirely novel pharmacological agents are also included. Educational items such as the allergy practice forum are of special interest to clinical allergists world wide.

Clinical Allergy was first published in 1971, the founding editor being Professor Jack Pepys. Between 1980 and 1983 the Journal was co-edited by Dr James Kerr and Dr William Parish. Most of the 1984 issues and the first two issues of 1985 were managed entirely by Dr Parish. The current co-editors are Professor Barry Kay and Professor Stephen Holgate who took over the editorship in 1985.

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milligram(s) (10^{-3}g)	mg
microgram(s) (10^{-6}g)	μg
nanogram(s) (10^{-9}g)	ng
picogram(s) (10^{-12}g)	pg
hours(s)	h
minute(s)	min
centimetre(s)	cm
second(s)	s
cubic millimetre(s)	mm^3
millimetre(s)	mm
millicurie(s)	mCi
milliequivalent	mmol
molar	mol l
millilitre(s)	ml
gravitational acceleration	*g*
micrometre(s)	μm
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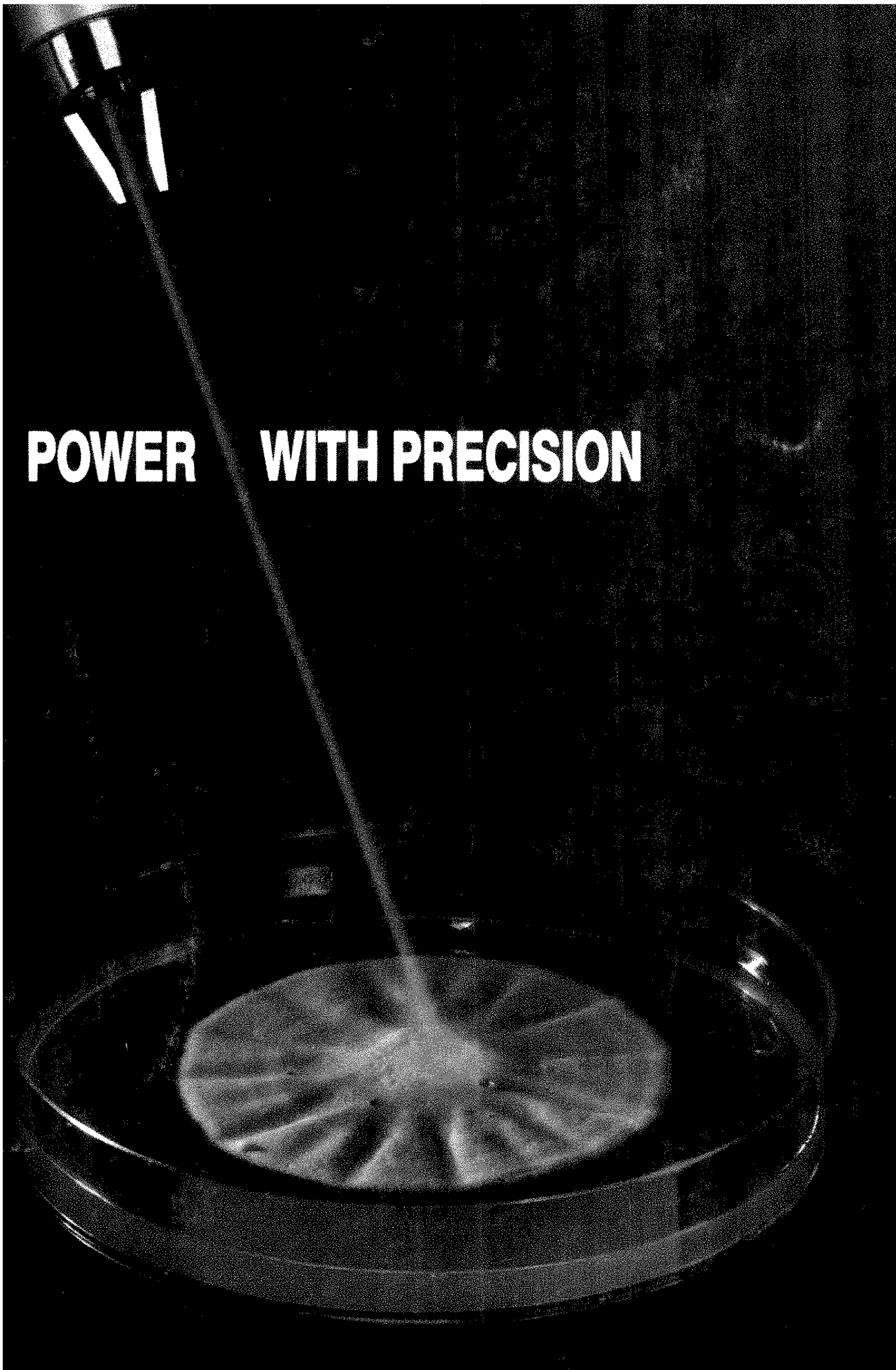

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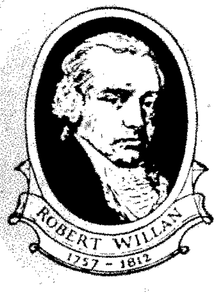

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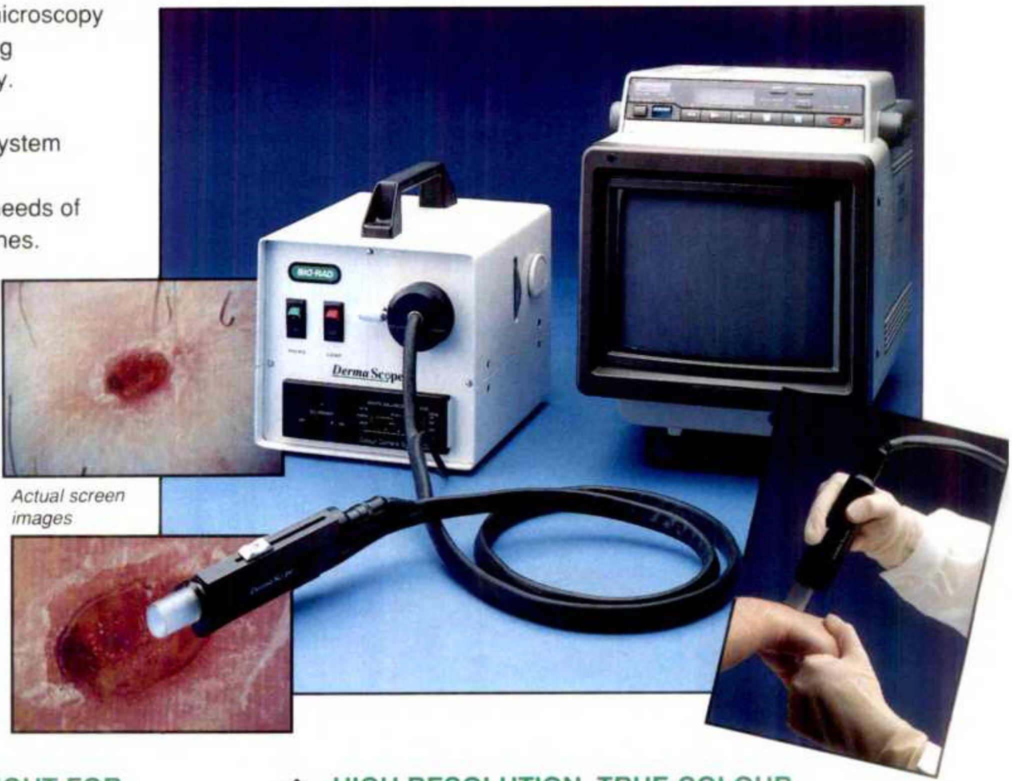
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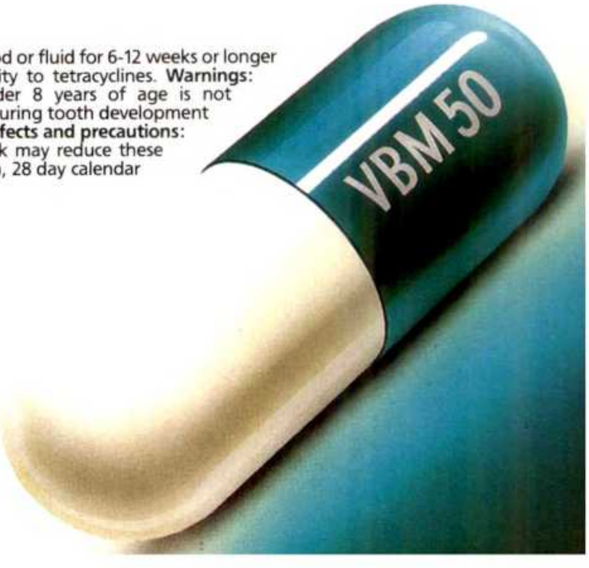

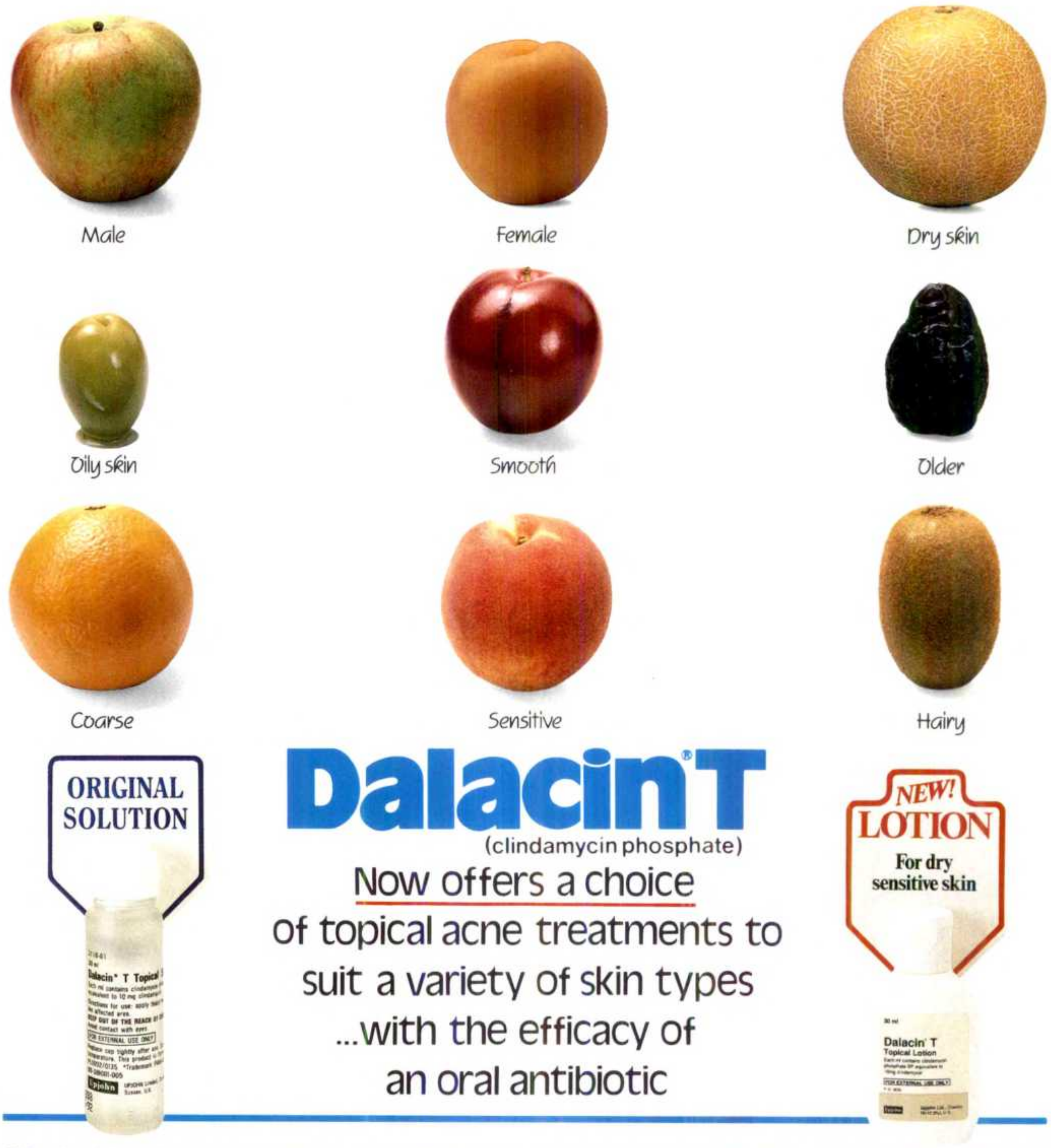

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REVIEW

Epidermal cytokines and their roles in cutaneous wound healing

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Summary Cytokines are small proteins or glycoproteins which are synthesized and secreted by a variety of cell types. Through binding to specific receptors on target cells, these hormone-like products regulate many normal cell activities, including growth and differentiation, migration and immune functions. Within the epidermis, keratinocytes are the major source of cytokines along with melanocytes and Langerhans cells. In response to a variety of injurious stimuli, including ultraviolet irradiation and cutaneous wounding, epidermal keratinocytes may release a number of these regulatory molecules which can then interact directly with receptors on inflammatory cells. Epidermal cytokines can therefore play an important role in the wound-healing process by recruiting polymorphs and monocytes and in encouraging deposition of extracellular matrix proteins by fibroblasts. Keratinocytes can themselves respond to keratinocyte-derived cytokines by dividing and migrating over the wound surface before differentiating into a new stratified epidermis. This review presents the evidence of the production of cytokines by human keratinocytes and their role in the healing of skin wounds.

In normal skin there is a balance between the number of keratinocytes being produced in the basal cell layer and the number of cells being shed at the skin surface, which results in a stable epidermis.[1] This in turn probably reflects a balance between growth stimulatory and growth inhibitory factors, now known as cytokines. When keratinocytes are grown *in vitro* they exhibit a hyperproliferative phenotype[2] and start to produce many of these cytokines.[3] Likewise cytokines can be found in abundance in skin which is inflamed or injured, especially in conditions with epidermal hyperproliferation such as psoriasis.[4–6] This has led to the concept of the 'activated keratinocyte':[7] a cell which in culture or in inflammatory skin disease releases a number of cytokines not found in the normal epidermis. Keratinocytes also appear to adopt such an activated phenotype during the process of wound healing. Below we review the cytokines produced by activated keratinocytes before proceeding to a description of their role in wound healing.

Keratinocyte-derived cytokines

Table 1 lists the cytokines shown to be produced by human keratinocytes, divided into three groups: growth factors, interleukins and colony stimulating factors. Most of them were originally identified by their biological activities and were given appropriate functional names which are listed alongside.

Table 1. Cytokines produced by human keratinocytes

		References
Growth factors		
bFGF	Basic fibroblast growth factor	8
PDGF AA and B	Platelet derived growth factor	9
TGF-α	Transforming growth factor α	4, 10, 11
TGF-β	Transforming growth factor β	11
TNF-α	Tumour necrosis factor α	9
Interleukins		
IL-1α and β	Epidermal cell-derived thymocyte activating factor (ETAF)	12, 13, 14
IL-6	Epidermal cell natural killer cell augmenting factor (ENKAF)	5, 15
IL-8	Neutrophil activating protein	16
Colony stimulating factors		
GM-CSF	Granulocyte-macrophage CSF	17
G-CSF	Granulocyte CSF	3
M-CSF	Macrophage CSF	18
Interleukin 3	Multi-CSF	19

Correspondence: Dr I.A.McKay.

Stimulatory growth factors

Transforming growth factor α (TGF-α) is found in normal epidermis[4,10,11] but, consistent with the concept of the 'activated keratinocyte',[7] elevated levels of TGF-α messenger RNA and protein are produced by keratinocytes in psoriatic epidermis[4,20,21]. This factor is synthesized as a large precursor molecule which is inserted into the keratinocyte membrane and on activation releases a 50-amino-acid peptide,[4] which will bind to the receptor for epidermal growth factor (EGF), normally found on the surface of basal keratinocytes.[22] Through this mechanism TGF-α induces keratinocyte division and stimulates keratinocyte migration *in vitro*,[23] thereby enlarging cell colonies. The fact that TGF-α has been shown to be a major autocrine growth factor for cultured keratinocytes has led to its testing along with related growth factors in wound healing. Recent evidence suggests that, despite its name, TGF-α does not cause transformation of keratinocytes[24] but seems to be the major positive factor involved in normal keratinocyte replication.

Basic fibroblast growth factor (bFGF) is produced by keratinocytes *in vitro* although it is not thought to be secreted by them. However it does stimulate the growth of melanocytes,[8] presumably acting either by direct cell-to-cell contact or by the binding of the growth factor to the extracellular matrix in some fashion. Bovine bFGF has been shown to stimulate human keratinocyte growth[25] and it is likely that human bFGF acts in a similar fashion. Certainly another member of the FGF family, KGF, acts as a mitogen for human keratinocytes.

The platelet-derived growth factors (PDGF) differ from bFGF and TGF-α in that keratinocytes produce them but lack receptors for them. PDGF derived from platelets exists as a disulphide-linked heterodimer of A and B chains. Cultured keratinocytes secrete both homodimers (AA and BB)[9] although they constitutively express more PDGF-A than PDGF-B. PDGF is a potent chemotactic factor as well as being an important mitogen for fibroblasts and it plays an important role in the development of granulation tissue during the wound-healing process.

Inhibitory growth factors

Factors produced by keratinocytes which inhibit their own growth include tumour necrosis factor α (TNF-α)[26] and transforming growth factor β (TGF-β).[27] TNF-α shows synergy with another inhibitory factor released by T cells, interferon γ (IFN-γ),[28] in suppressing keratinocyte growth and increasing the expression of intercellular adhesion molecules. TGF-β, a 25-kDa protein, is a very important regulator of inflammation involved both in recruiting macrophages and inhibiting some lymphocyte functions.[29]

Interleukins

Interleukins constitute a class of cytokines originally identified for their role in mediating immunological functions.[30] In recent years it has been shown that keratinocytes produce or respond to a number of interleukins, indicating that keratinocytes themselves are important members of the skin immune system.

Interleukin 1 is produced by keratinocytes in two forms, IL-1α and IL-1β.[13] Kupper[7] has suggested that the IL-1 found in keratinocytes can only be released once the cell is injured. Neither form is usually secreted but both require activation by serine proteases for their function. Keratinocytes have IL-1 receptors on their surface and it is likely that IL-1 can activate keratinocytes *in vivo* through an autocrine pathway.

Interleukin 6 (IL-6) is synthesized as a 26-kDa peptide which can be secreted from cells in multiple glycosylated forms. *In-situ* hybridization experiments show the mRNA for IL-6 in the normal epidermis[5] and, like TGF-α, it is found in elevated quantities in psoriasis and in cultured keratinocytes and skin tumour-cell lines.[5,15] Like IL-1, IL-6 is a major mediator of the acute-phase response.[31] It is released from injured cells following bacterial and viral infections, UV-irradiation and burns and is found in elevated levels in the sera of patients following surgery.[32,33]

Thus both IL-1 and IL-6 are products of the 'activated keratinocyte'.[7] As yet there is little evidence of a role for these factors in wound healing, although it seems likely that they will soon be implicated in the activation of inflammatory cells and in the replication of keratinocytes during this process.

Interleukin 8 (IL-8), also known as neutrophil activating protein (NAP),[16] belongs to a family that includes β-thromboglobulin, which mediates both growth stimulatory and inflammatory processes.[34] IL-8 is produced by cultured human dermal fibroblasts and keratinocytes in response to IL-1 or TNFα.[16]

Colony stimulating factors

The colony stimulating factors (CSFs) support the formation of different blood-cell colonies from haemopoietic precursor cells in semi-solid media. Four colony stimulating factors are produced by cultured keratinocytes:[3] multi-CSF (IL-3),[19] granulocyte-CSF (G-CSF),[3] macrophage-CSF (M-CSF)[18] and granulocyte/macrophage-CSF

(GM-CSF).[17] The best characterized in the skin is GM-CSF, a 15-kDa protein, which activates mature granulocytes, eosinophils and T cells as well as promoting granulocyte and macrophage differentiation in the bone marrow. There is some evidence that GM-CSF may also play an important role in the maturation of Langerhans cells.

Keratinocytes respond to cytokines produced by other cells

Cytokines which have effects on human keratinocytes but are produced by other cell types and not keratinocytes are listed in Table 2.

Epidermal growth factor (EGF) was the first cytokine shown to have any effect on the keratinocyte. Although EGF binds to receptors in human keratinocytes *in vitro* and promotes both their growth and persistence, it is not found in any significant quantities in normal human epidermis. However, EGF is found in the α granules of platelets and may be released during wound healing.[38]

Other cytokines released from inflammatory cells and fibroblasts, blood mononuclear cells and T cells affect keratinocytes in various disease states. These cytokines include KGF[36] (keratinocyte growth factor), a member of the fibroblast growth factor family but with specificity for epithelial cells, IFN-γ[28] and IL-4.[35] Interferons α, $\beta 1$[37] and γ,[28] like TNF-α, reversibly arrest the growth of keratinocytes in culture, whereas KGF is mitogenic.[36] The final effect of cytokines on the growth of keratinocytes clearly depends on a balance between positive and negative factors acting synergistically or antagonistically.

Activated keratinocytes in wound healing

Any damage to keratinocytes by ultraviolet irradiation, thermal burns, bacterial and viral infections or incisional or excisional wounding activates the injured keratinocytes. Activated keratinocytes may then release variety of factors like IL-1, IL-6 and TGF-α which can bind to receptors on a variety of different inflammatory cells, including the keratinocytes themselves. Activation of those cells in turn may result in the transcription of genes encoding other cytokines such as TNF-α, IL-8 and GM-CSF and their subsquent release in biologically active forms.

Table 2. Cytokines produced by other cell types which have effects on human keratinocytes

Factor		Reference
IL-4	Interleukin 4	35
KGF	Keratinocyte growth factor (FGF7)	36
IFN-α	Interferon alpha	37
IFN-$\beta 1$	Interferon beta 1	37
IFN-γ	Interferon gamma	28
EGF	Epidermal growth factor	23
TNF-β	Lymphotoxin	28

Keratinocytes may also become activated by other pathways, possibly dependent on IFN-γ release from T cells or TNF-α from monocytes. The nature of the inflammatory infiltrate may determine the degree of keratinocyte activation and resulting level of cytokine induction. Multiple keratinocyte activators, IL-1, IFN-γ and TNF-α have been identified and it is possible that one or more of these factors released from damaged keratinocytes is responsible for initiating the process of wound healing.

The role of cytokines in wound healing

The healing of skin wounds can be divided into four phases all potentially involving the mediation of cytokines.

Inflammation. This involves the release of agents chemotactic for capillary endothelial cells and cells of the immune system, such as lymphocytes, polymorphs and macrophages.

Migration. As epidermal epithelial cells (keratinocytes) migrate from hair follicles and from the wound edge towards the centre of the wound, immune cells and capillaries migrate through the underlying dermis into the wound site.

Deposition. Matrix components such as the collagens, laminin and fibronectin are deposited in the dermis by fibroblasts and other cell types.

Maturation. This involves the contraction of the dermis in full-thickness wounds and squamous differentiation of the keratinocytes on the wound surface.

The study of the role of cytokines in wound healing has been subject to the availability of cytokines in pure form. Initially this involved laborious isolation of proteins from cell extracts and supernatants etc. but more recently, pure factors have been produced by recombinant DNA technology. Early studies focused on the use of EGF[39,40] but TGF-α, vaccinia growth factor (VGF),[41] bFGF[42] and PDGF[43] have been tested more recently. Crude biological extracts such as platelet releasates[44] and homogenates or grafts of cultured keratinocytes[45] have been used as sources of bioavailable growth factors but their net result depends on the synergism or antagonism of the contained factors. Most of these studies have been performed in animal models as difficulties have been reported with the use of recombinant products on human volunteers.

There are problems in the assessment of many of the available studies due to variations in the following factors.

The animal model. Initial experiments were performed on mice or on human volunteers but now the pig has re-emerged as the model of choice, both because of the acknowledged similarities between the pig and humans and because the larger wounds can be more easily surveyed during the healing process.

The method of applying the cytokines. Cytokines have been applied as wound irrigation in buffered solution and as ointments but the long-term stability and activity of these cytokines has seldom been established.

The method of assessing wound healing. The measurement of wound area is complicated by the damage from repeated dressings, difficulties in accurate assessment of the wound edge and distortion from wound contraction.

Mimicking of chronic wounds. Many of the clinical problems of wound healing involve chronic non-healing wounds such as leg ulcers and burns but these are very difficult to mimic in animal models. Steroid treated and diabetic (induced) animals have been used but cannot reproduce the underlying vascular problems of a chronic leg ulcer.

Results of wound healing studies to date

Despite slow and intermittent progress, the results of cytokine testing on animal and human wounds parallel the results on keratinocytes *in vitro.* EGF and TGF-α promote keratinocyte growth and migration both in culture and in wounds.[23,39,40] The application of a saline solution of mouse EGF to suction blisters did not significantly affect epidermal regeneration[39] but healing of split-thickness wounds on pig flank was enhanced by application of EGF in a cream vehicle.[40] Similarly, on human volunteers EGF in Silvadene was found to be superior to vehicle alone in 12 donor sites with enhanced epidermal regeneration and no complications at 1 year following application.[44] More recently, TGF-α and VGF improved the healing of split thickness incisional wounds and partial thickness burns but this study was limited to two pigs.[41]

In pig wounds, TGF-β application caused an increase in connective tissue including elevated collagen levels, a promotion of angiogenesis and an increase in tensile strength,[29] but TGF-β is pro-inflammatory and causes abnormal epidermal differentiation.

It has been suggested that limited effects from the use of individual factors might be overcome by their application in combinations. There have been few studies of this type. Eisinger *et al.* used extracts of cultured keratinocytes and supernatants from cultured cells on split-thickness wounds in pigs.[45] Five days after application there was more extensive epidermal regeneration in the treated wounds when compared with controls. It seems probable however that this form of treatment has no advantages over the application of cultured skin grafts, which presumably release the same factors but are likely have a superior dressing effect. Lynch *et al.*[46] tested the effects of various combinations of purified cytokines on pig wounds. They reported that the combinations of PDGF with either insulin-like growth factor 1 (IGF-1) or TGF-α gave the best results in terms of connective tissue deposition, angiogenesis and collagen content and maturity. Indeed, compared to TGF-β alone, these combinations of factors also give better epidermal differentiation and reduced inflammation. It seems therefore that combinations of factors will be attractive alternatives to grafting or single factor therapies. Studies on the effects of the interleukins and colony stimulating factors on wound healing are so far lacking. However it seems likely that potent activators of keratinocytes such as IL-1 will have an important role to play in the wound healing process.

The future

In wound healing studies the challenge of the nineties will be to ascertain which cytokine activities are important. The tools are readily available for such studies: monospecific antisera for immunocytochemistry, cloned DNA probes for *in-situ* hybridization and biological assays of function. Using this powerful combination it should prove possible to discover which cytokines are released in the initial response to cutaneous wounds; the pattern of cytokines subsequently induced; how much of each cytokine is present and how long they remain active. Only with this information will it be possible to define rational therapeutic approaches to intervention in the would healing process. Meanwhile the cost of recombinant factors excludes their use in routine wound healing management. However, large-scale manufacture of stable active formulations may allow their use in treatment of chronic non-healing wounds or hypertrophic scars.

Acknowledgments

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References

1 Bullough WS. The control of mitotic activity in adult mammalian tissues. *Biol Rev* 1962; 37: 307–42.

2 Leigh IM, Pulford KA, Ramaekers FCS, Lane EB. Psoriasis: maintenance of an intact monolayer basal cell differentiation compartment in spite of hyperproliferation. *Br J Dermatol* 1985; 113: 53–64.

3 Kupper TS, Horowitz M, Birchall N *et al.* Hematopoietic, lymphopoietic and proinflammatory cytokines produced by human and murine keratinocytes. In: *Endocrine, Metabolic and Immunologic Functions of Keratinocytes* (Milstone LM, Edelson RL, eds), Annals NY Acad Sci, Vol. 548. New York Academy of Sciences Publications, 1988; 262–70.

4 Gottlieb AB, Chang CK, Posnett DN *et al.* Detection of transforming growth factor α in normal, malignant and hyperproliferative human keratinocytes. *J Exp Med* 1988; 167: 670–5.

5 Grossman RM, Krueger J, Yourish D *et al.* Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. *Proc Natl Acad Sci USA* 1989; 86: 6367–71.

6 Krueger JG, Krane JF, Carter M *et al.* Role of growth factors, cytokines and their receptors in the pathogenesis of psoriasis. *J Invest Dermatol* 1990; 94: 135S–40S.

7 Kupper TS. The activated keratinocyte: a model for inducible cytokine production by non-bone marrow-derived cells in cutaneous inflammatory and immune responses. *J Invest Dermatol* 1990; 94: 146S–50S.

8 Halaban R, Langdon R, Birchall N *et al.* Basic fibroblast growth factor from human keratinocytes is a natural mitogen for melanocytes. *J Cell Biol* 1988; 107: 1611–19.

9 Ansel J, Perry P, Brown J *et al.* Cytokine modulation of keratinocyte cytokines. *J Invest Dermatol* 1990; 94: 101S–7S.

10 Coffey RJ Jr, Derynck R, Wilcox JN *et al.* Production and autoinduction of transforming growth factor-α in human keratinocytes. *Nature* 1987; 328: 817–20

11 Partridge M, Green MR, Langdon JD, Feldmann M. Production of TGF-α and TGF-β by cultured keratinocytes, skin and oral squamous cell carcinomas—potential autocrine regulation of normal and malignant epithelial cell proliferation. *Br J Cancer* 1989; 60: 542–48.

12 Luger TA, Stadler BM, Luger BM *et al.* Characteristics of an epidermal cell thymocyte-activating factor (ETAF) produced by human epidermal cells and a human squamous cell carcinoma line. *J Invest Dermatol* 1983, 81: 187–93.

13 Kupper TS, Ballard DW, Chua AO *et al.* Human keratinocytes contain mRNA indistinguishable from monocyte interleukin 1α and β mRNA. *J Exp Med* 1986; 164: 2095–100.

14 Ristow H-J. A major factor contributing to epidermal proliferation in inflammatory skin diseases appears to be interleukin 1 or a related protein. *Proc Natl Acad Sci USA* 1987; 84: 1940–4

15 Kirnbauer R, Kock A, Schwarz T *et al.* IFNβ2, B cell differentiation factor 2 or hybridoma growth factor (IL-6) is expressed and released by human epidermal cells and epidermoid carcinoma cell lines. *J Immunol* 1989; 142: 1922–8.

16 Larsen CG, Anderson AO, Oppenheim JJ *et al.* Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumour necrosis factor. *Immunology* 1989; 68: 31–6.

17 Kupper TS, Lee F, Coleman D *et al.* Keratinocyte derived T-cell growth factor (KTGF) is identical to granulocyte macrophage colony stimulating factor (GM-CSF). *J Invest Dermatol* 1988; 91: 185–8.

18 Chodakewitz JA, Lacy J, Edwards SE *et al.* Macrophage colony-stimulating factor production by murine and human keratinocytes. *J Immunol* 1990; 144: 2190–6.

19 Luger TA, Kock A, Kirnbauer R *et al* Keratinocyte-derived interleukin 3. In. *Endocrine, Metabolic and Immunologic Functions of Keratinocytes* (Milstone LM, Edelson RL, eds), Annals NY Acad Sci, Vol. 548. New York Academy of Sciences Publications, 1988; 253–61.

20 Elder JT, Fisher GJ, Lindquist PB *et al.* Overexpression of transforming growth factor α in psoriatic epidermis. *Science* 1989; 243: 811–14.

21 Turbitt ML, Akhurst RJ, White SI, MacKie RM. Localization of elevated transforming growth factor-alpha in psoriatic epidermis. *J Invest Dermatol* 1990; 95: 229–32

22 King LE Jr, Gates RE, Stoscheck CM, Nanney LB. The EGF/ TGFα receptor in skin. *J Invest Dermatol* 1990; 94: 164S–70S.

23 Barrandon Y, Green H. Cell migration is essential for sustained growth of keratinocyte colonies; the roles of transforming growth factor and epidermal growth factor. *Cell* 1987; 50: 1131–7.

24 Finzi E, Fleming T, Pierce JH. Retroviral expression of transforming growth factor-alpha does not transform fibroblasts or keratinocytes. *J Invest Dermatol* 1990; 95: 382–7.

25 O'Keefe EJ, Chiu ML, Payne RE. Stimulation of growth of keratinocytes by bFGF. *J Invest Dermatol* 1988; 90: 767–9.

26 Pillai S, Bikle DD, Eessalu TE *et al.* Binding and biological effects of tumour necrosis factor alpha on cultured human neonatal foreskin keratinocytes. *J Clin Invest* 1989; 83: 816–21.

27 Shipley GD, Pittelkow MR, Wille JJ Jr *et al.* Reversible inhibition of human prokeratinocyte proliferation by type beta transforming growth factor inhibitor in serum free medium. *Cancer Res* 1986; 46: 2068–71.

28 Symington FW. Lymphotoxin, tumor necrosis factor and gamma interferon are cytostatic for normal human keratinocytes. *J Invest Dermatol* 1989; 92: 798–805.

29 Quaglino D Jr, Nanney LB, Kennedy R, Davidson JM. Transforming growth factor-β stimulates wound healing and modulates extracellular matrix gene expression in pig skin. *Lab Invest* 1990, 63: 307–19.

30 O'Garra A, Umland S, De France T, Christiansen J 'B-cell factors' are pleiotropic. *Immunol Today* 1988; 9: 45–54.

31 Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. *Biochem J* 1990; 265: 621–36.

32 Nijsten MWN, De Groot ER, Ten Duis HJ. Serum levels of IL-6 and acute phase responses. *Lancet* 1987; ii: 921.

33 Nishimoto N, Yoshizaki K, Tagoh H *et al.* Elevation of serum interleukin 6 prior to acute phase proteins on the inflammation by

surgical operation. *Clin Immunol Immunopathol* 1989, **50**: 399–401.

34 Stoeckle MY, Barker KA. Two burgeoning families of platelet factor 4-related proteins: mediators of the inflammatory response *New Biologist* 1990; **2**: 313–23.

35 Birchall N, Park L, Kupper T. IL-4 binds to specific cell surface receptors on human keratinocytes and induces colony stimulating factor release and HLA-DR expression. *Clin Res* 1988; **36**: 374A.

36 Finch PW, Rubin JS, Miki T *et al.* Human KGF is FGF-related with properties of a paracrine effector of epithelial cell growth. *Science* 1989; **245**: 752–5.

37 Yaar M, Karassik RL, Schnipper LE, Gilchrest BA. Effect of alpha and beta interferon on cultured human keratinocytes. *J Invest Dermatol* 1985; **85**: 70–4.

38 Ben-Ezra J, Sheibani K, Hwang DL, Lev-Ran A. Megakaryocyte synthesis is the source of epidermal growth factor in human platelets. *Am J Pathol* 1990; **137**: 755–9.

39 Greaves MW. Lack of effect of topically applied epidermal growth factor (EGF) on epidermal growth in man *in vivo*. *Clin Exp Dermatol* 1980; **5**: 101–3

40 Brown GL, Curtsinger L, Brightwell JR *et al.* Enhancement of epidermal regeneration by biosynthetic epidermal growth factor. *J Exp Med* 1986; **163**: 1319–24.

41 Schultz GS, White M, Mitchell R *et al.* Epithelial wound healing enhanced by transforming growth factor-α and vaccinia growth factor. *Science* 1987; **235**: 350–2.

42 Schweigerer L. Basic fibroblast growth factor as a wound healing hormone. *TIPS* 1988; **9**: 427–8.

43 Gills JP, McIntyre LG. Growth factors and their promising future. *J Am Optom Assoc* 1989; **60**: 442–5.

44 Van Brunt J. Lessons from wound healing clinical trials. *Biotechnology* 1989; **7**: 15–16.

45 Eisinger M, Sadam S, Silver IA, Flick RB. Growth regulation of skin cells by epidermal cell-derived factors: Implications for wound healing. *Proc Natl Acad Sci USA* 1988; **85**: 1937–41.

46 Lynch SE, Colvin RB, Antoniades HN. Growth factors in wound healing. *J Clin Invest* 1989; **84**: 640–6.

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Modulation of leucocyte adhesion molecules, a T-cell chemotaxin (IL-8) and a regulatory cytokine (TNF-α) in allergic contact dermatitis (rhus dermatitis)

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Summary

To understand the molecular events which are important in leucocyte trafficking in cutaneous inflammation, poison ivy/oak extract was applied topically to the skin, and the simultaneous assessment of a variety of clinical and immunopathological parameters performed. The clinical response of subjects was divided into three main groups: I, 2–24 h after application, before the onset of erythema; II, 48 h–1 week after application during maximal clinical changes; III, 2–3 weeks after application when the inflammation had subsided. Six different biopsies per subject were evaluated over the study period and the density of dermal cellular infiltrate, and the distribution of intercellular adhesion molecule-1, (ICAM-1), endothelial leucocyte adhesion molecule-1, (ELAM-1), vascular cell adhesion molecule-1, (VCAM-1), interleukin 8 (IL-8) and tumour necrosis factor-alpha (TNF-α), determined. Eight hours after exposure, before lymphocytes and monocytes had entered the dermal interstitium or epidermis, the keratinocytes expressed TNF-α and ICAM-1, whilst the endothelial cells expressed ELAM-1, VCAM-1 and ICAM-1. Group II biopsies revealed increasing keratinocyte expression of TNF-α and ICAM-1 with the appearance of IL-8, which correlated with the onset of epidermal T-cell trafficking. The endothelium was strongly positive for ELAM-1 and VCAM-1, but there was no influx of neutrophils. Group III biopsies showed a decrease in the expression of ICAM-1, VCAM-1 and ELAM-1 by both keratinocytes and endothelium with a reduction in epidermal/dermal inflammation, although the endothelial cell staining of VCAM-1 and ELAM-1 did not completely disappear. These results suggest that on exposure to poison ivy/oak, keratinocytes rapidly produce TNF-α which leads to an early autoinduction of ICAM-1, and later IL-8. There is also a paracrine-mediated induction and augmentation of underlying endothelial cell ELAM-1, VCAM-1 and ICAM-1.

The cellular events induced in human skin following epicutaneously applied allergens include early morphological changes in endothelial cells, within 4–8 h, which precede the migration of lymphocytes into the dermis.[1] However, after 24–48 h, lymphocytes are observed bound to endothelial cells of the superficial vascular plexus, and entering the dermis and epidermis.[1] These cellular alterations imply that resident cells, keratinocytes, endothelial cells and perivascular dermal dendrocytes, can become rapidly activated and produce molecular mediators for the recruitment of circulating mononuclear cells into the dermis/epidermis. We previously observed that urushiol (the active ingredient in poison ivy/oak) directly induces keratinocyte ICAM-1, prior to dermal T-cell infiltration, with dermal dendrocytes appearing as prominent early constituents of the inflammatory reaction.[2]

To define the molecular events responsible for the migration of inflammatory cells, we have applied a poison ivy/oak extract to normal skin and taken sequential biopsies.[2] A variety of leucocyte adhesion molecules (ICAM-1, ELAM-1, VCAM-1), a T-cell chemotactic factor (IL-8), and a cytokine (TNF-α), known to regulate adhesion molecule and IL-8 production in co-ordination,[3] were assessed immunohistochemically. At the same time the sequence of trafficking of inflammatory cells in the skin during the evolution of inflammation was determined.[3,4]

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Methods

Patients

Seven adult volunteers (four males, three females; age range 26–54 years) with a positive history for poison ivy dermatitis, who were otherwise in good health without any current skin disorder, and not taking any medication, were selected for study. All 4-mm punch biopsies were obtained after informed consent with the approval of the University of Michigan Human Subjects Committee. Portions of a tonsil were also obtained from a patient undergoing elective tonsillectomy.

Induction of inflammation and biopsy procedure

A poison ivy/oak mixture 1:50 (w:v) in alcohol (Hollister-Stier, Elkhart, IN, U.S.A.) was applied using the Al-test System (Sodertalje, Sweden) to six different sites (5 mm diameter, 5 cm apart) on the flexor forearm of each volunteer. The patch-test reaction sites were divided into three different groups.[2] Group I biopsies were obtained 2–24 h after application of the poison ivy/oak mixture, before the onset of erythema or any other detectable clinical change. Group II biopsies were obtained 48 h–1 week after application when there were maximal clinical changes, and Group III biopsies were obtained 2–3 weeks after application when the inflammation had subsided, and there was only mild, residual erythema. Each volunteer had a baseline (pre-application) biopsy and then sequential biopsies of Group I, Group II and Group III sites were taken, with no more than six biopsies taken from each subject. A representative biopsy protocol would be as follows: baseline; 4, 8, and 24 h (Group I); 48 h or 1 week (Group II); 2 or 3 weeks (Group III).

Evaluation procedures

Clinical features. The patch-test sites were graded as follows: 0, no reaction; 1, erythema; 2, erythema and oedema; 3, erythema, oedema, and vesiculation.

Histopathology. The slides were assessed by two of the authors (CEMG, BJN). The dermal cellular infiltrate was composed primarily of lymphocytes and monocytes/macrophages (without neutrophils) and was graded semiquantitatively from 0–4 as follows: 0–1, no increase above the normal, pretreatment biopsy, or a few perivascular mononuclear cells; 2, a mild increase in perivascular mononuclear cells with rare scattered interstitial cells; 3, a moderate increase in perivascular cells accompanied by an easily recognizable dermal interstitial and epidermal influx of mononuclear cells and 4, an intense perivascular and interstitial mononuclear cell infiltrate with numerous intraepidermal cells.

Immunohistochemistry. The punch biopsies were orientated and mounted on gum tragacanth (Sigma Chemical Co., St Louis, MO, U.S.A.), snap frozen in isopentane chilled in liquid nitrogen, and then stored at −70°C until use. Cryostat sections (5 μm) were fixed in cold (4°C) acetone and incubated with primary monoclonal antibodies (MAbs) or antisera diluted in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (Sigma Chemical Co.) for 45 min at room temperature. The sections were then washed in PBS, and an avidin–biotin immunoperoxidase technique was used (Vectastain, Vector Labs, Burlingame, CA, U.S.A.) as previously described,[2] with 3-amino 9-ethylcarbazole as the chromogen and counterstained with 1% haematoxylin.

The MAbs used were BB11 (anti-ELAM-1) and RR1/1 (anti-ICAM-1) obtained from Drs R. Lobb and C. Benjamin (Biogen Inc., Boston, MA, U.S.A.) and Dr T. Springer (Dana Farber Cancer Institute, Boston, MA, U.S.A.), respectively; MAb 4B9 (anti-VCAM-1) was produced as previously described.[5] Staining of human umbilical vein endothelial cells (obtained courtesy of Dr R. Marks, University of Michigan) before and after exposure to TNF-α (250 U/ml; Biogen Inc.) for 6 and 24 h using these anti-ELAM-1 and anti-VCAM-1 antibodies produced identical results to those previously published (data not shown).[6,7] The highly purified rabbit antisera against human TNF-α and IL-8 was provided by Dr S.Kunkel. The sensitivity and the specificity of the rabbit anti-TNF-α and IL-8 sera was confirmed by comparison to pre-immune rabbit sera, as well as neutralization pre-incubation studies with recombinant TNF-α and IL-8 as previously described.[8] Other MAbs used included Leu 6 (anti-CD1, Becton Dickinson, Burlingame, CA, U.S.A.), T11 (anti-CD2, a pan-T cell marker, Coulter Immunology, Hialeah, FL, U.S.A.), and EBM 11 (anti-monocyte/macrophage; Dako Corp, Santa Barbara, CA, U.S.A.). The specific dilution and antibody concentrations used were: BB11 (1:50; 20 μg/ml); 4B9 (1:50; 20 μg/ml); RR1/1 (1:10,000; 1 μg/ml); TNF-α and IL-8 (1:2000); Leu 6 (1:20; 2·5 μg/ml) and T 11 (1:50; 10 μg/ml); EBM 11 (1:100; 4 μg/ml).

Keratinocyte ICAM-1 expression was graded as: 0, no epidermal staining; 1, less than 25% of all epidermal keratinocytes/section were positive; 2, 25–50%; 3, 50–75%; 4, >75%. The same scoring system was used to measure the degree of epidermal keratinocyte TNF-α and IL-8 positivity.

Endothelial cell adhesion molecule (ELAM-1, ICAM-1 and VCAM-1) expression was assessed by the following scoring system: 0, negative or no vessel reactivity; 2, weak reactivity that was generally detectable on less than five vessels per section; 4, moderate reactivity easily detectable on 6–10 vessels per section; 6, strong reactivity with easily detectable and diffuse staining of more than 10 vessels per section. It should be noted that the positively scored vessels were primarily those of the superficial vascular plexus and not larger vessels (arteriolar) or lymphatic endothelium.

Results

Normal skin and tonsil

The baseline biopsies of normal skin revealed no reactivity for ELAM-1 and VCAM-1 on endothelial cells and only weak ICAM-1 staining of endothelial cells as previously described.[2,6,7] Perivascular dermal dendritic cells, and occasional perivascular lymphocytes were positive for VCAM-1. The perivascular dendritic cells which were VCAM-1 positive were in the same position as those labelled by the macrophage antibody EBM 11, but there was no labelling of epidermal CD1$^+$ Langerhans cells (data not shown). The follicular dendritic cells within the central portion of the lymphoid follicles, and the interdigitating dendritic cells in the interfollicular zones were strongly VCAM-1 positive, as found by Rice *et al.* using the INCAM-110 MAb that recognizes VCAM-1.[7] Fewer than 10% of the lymphocytes in the T-cell and B-cell zone of the tonsillar tissue were VCAM-1 positive, and only occasional venular endothelial cells were VCAM-1 positive. The venular endothelium of the tonsil was focally ELAM-1 positive, but there was no staining of arterioles, or any other cell types (data not shown). Perivascular cutaneous dermal dendrocytes and eccrine

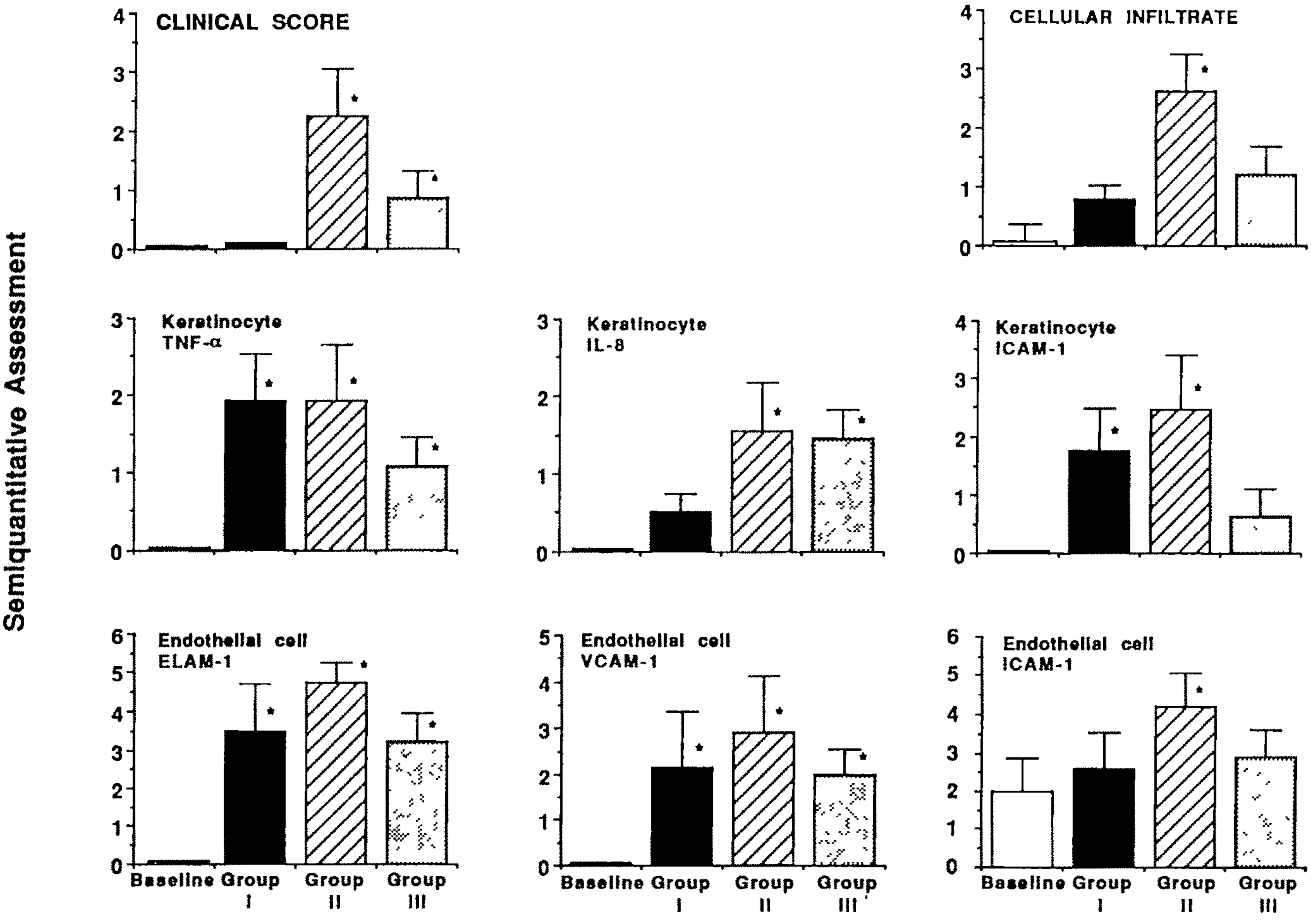

Figure 1. Summary of clinical, pathological, and immunohistochemical results including a semiquantitative assessment of staining of TNF-α, IL-8, ICAM-1, ELAM-1 and VCAM-1 (means+SD) * $P<0{\cdot}05$ when compared to baseline valves using the non-parametric sign test

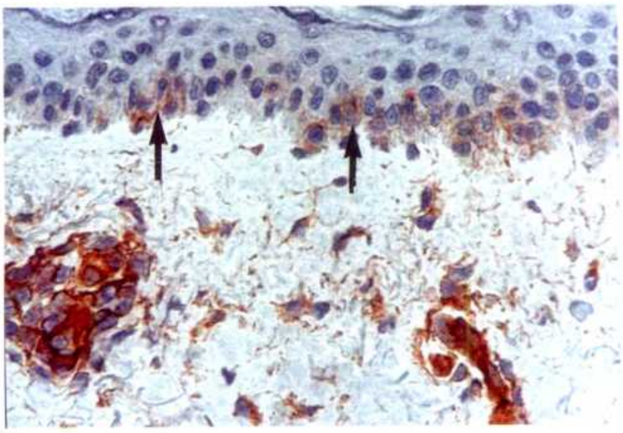

Figure 2. ICAM-1 expression within 24 h of application of poison ivy/ oak extract by basal keratinocytes (straight arrows) and by dermal dendritic cells located in the upper dermis.

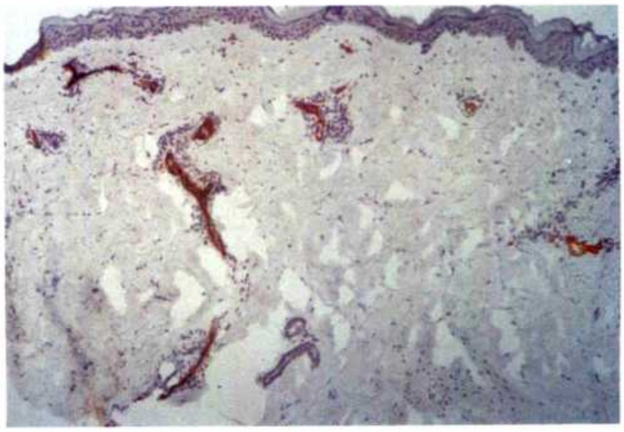

Figure 3. ELAM-1 expression on endothelial cells in both the superficial and deep vascular plexus 8 h after treatment.

sweat duct epithelium were positive for TNF-α. IL-8 positive cells were confined to eccrine duct epithelium and acrosyringium.[8] This IL-8 and TNF-α reactivity in normal skin, as well as the staining pattern described below was judged to be specific as it was not observed using pre-immune rabbit serum, and pre-absorption of the anti-serum with recombinant IL-8 and TNF-α resulted in an absence of staining.[8] A summary of the clinical, pathological and immunohistochemical results for pretreatment and post-treatment biopsies is shown in Figure 1.

Group I: pre-erythematous skin reactions to poison ivy/oak

During the first 24 h following application of poison ivy/ oak, the skin was essentially clinically unchanged, with only a slight degree of erythema detected in one patient (Fig. 1) The cellular infiltrate during this period was only slightly increased over baseline levels, with a marginal increase in the perivascular lymphocyte population (Fig. 1) but with no detectable movement of lymphocytes into the surrounding dermis.[1,2] However, there was a significant increase in the keratinocyte expression of ICAM-1

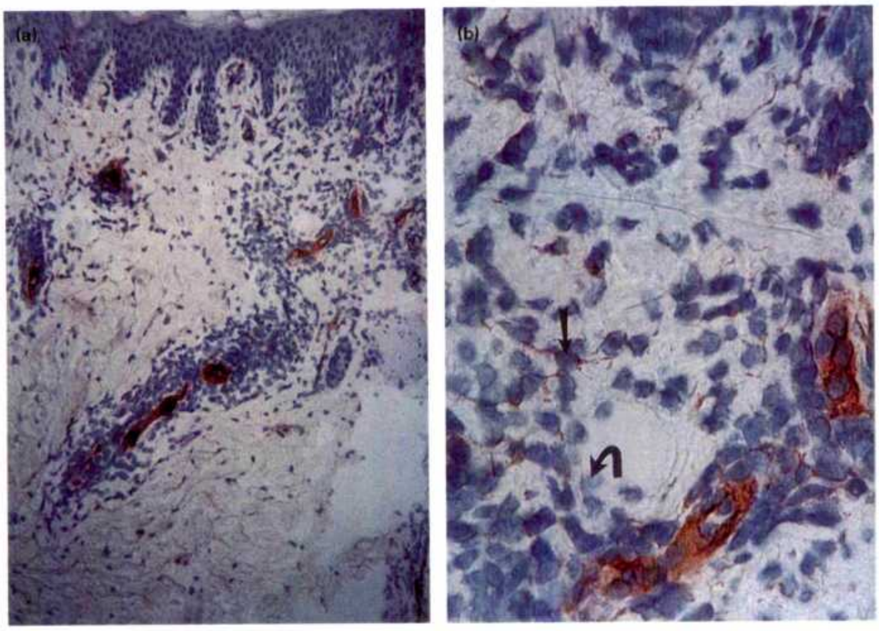

Figure 4. VCAM-1 expression on vascular endothelial cells and perivascular dendritic cells (straight arrow) 18 h after treatment (a) low-power magnification; (b) higher power magnification. Note the absence of VCAM-1 expression by lymphatic endothelium (curved arrow).

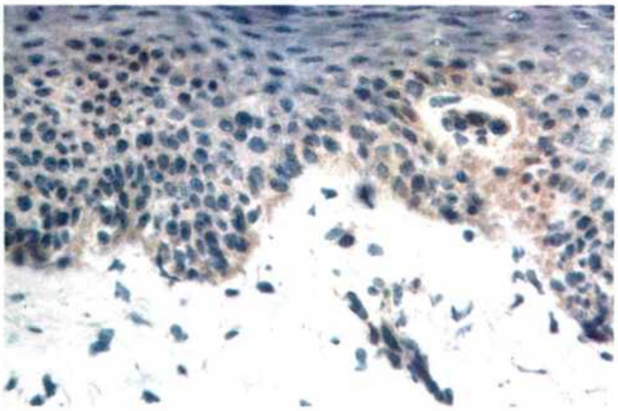

Figure 5. Expression of TNF-α by epidermal keratinocytes as early as 4 h after treatment, with enhancement along the basement membrane zone.

and more prominent ICAM-1 expression of dermal dendritic cells in the upper dermis, particularly in the area between the tips of the dermal vascular plexus and the base of the keratinocytes (Fig. 2). It appeared that these dermal dendritic cells were more strongly ICAM-1 positive than dermal dendritic cells in the normal and untreated skin.[9] The prominent expression of ICAM-1 by basal keratinocytes, dermal dendrocytes, and the endothelial cells seemed to produce an almost continuous network of cellular adhesion molecules in the upper portion of the treated skin, which probably comprised a dermal microvascular reactive unit.[10] The endothelial cells of the superficial vascular plexus became positive for both ELAM-1 (Fig. 3) and VCAM-1 (Fig. 4) during this period and this could be detected as early as 4 h after application of the poison ivy/oak extract.

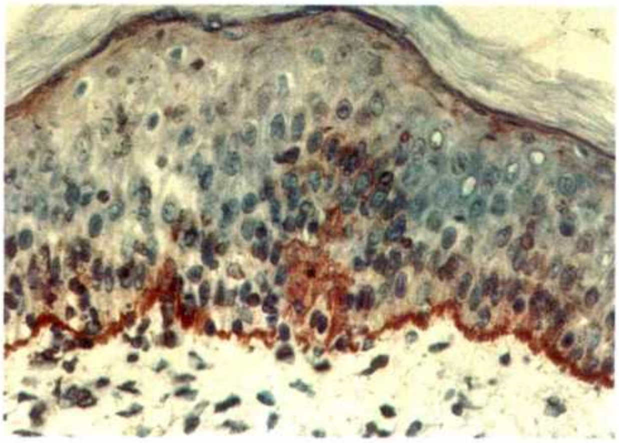

Figure 6. IL-8 expression by epidermal keratinocytes 48 h after treatment, accompanied by the movement of lymphocytes into the epidermis.

Because there was a near-simultaneous induction of the expression of ICAM-1 by keratinocytes and of ELAM-1 and VCAM-1 by endothelial cells, suggesting the local production of an activating cytokine,[11] we sought to identify TNF-α in these sections. TNF-α is the only known cytokine which can induce all three adhesion molecules on these different cell types *in vitro*.[12–14] The epidermal keratinocytes showed an increased expression of TNF-α during this time period with little or no staining of any other cell type (Fig. 5). There was only a slight increase in keratinocyte IL-8 expression, which became more noticeable 48 h after treatment (Fig. 6).

Group II: inflammatory phase of poison ivy/oak reaction

Two days to 1 week following application of poison ivy/oak, the skin became erythematous with oedema and vesiculation. There was variation between patients in the degree and time of onset for each of these parameters,[2] but all patients eventually reached a clinical score of at least 2·0. The cellular infiltrate was greatly increased with prominent perivascular and interstitial dermal collections of lymphocytes, monocytes and macrophages, but no neutrophils. Mononuclear cells also began entering the epidermal compartment, with concomitant spongiosis and microvesiculation in four cases. The dermal and epidermal lymphocytes were negative for ELAM-1 and VCAM-1. Epidermal keratinocyte ICAM-1 expression gradually increased, as did endothelial staining for ICAM-1, ELAM-1 and VCAM-1. There was a slight decrease in keratinocyte TNF-α staining, but increased keratinocyte IL-8 staining as noted above (Fig. 6).

Group III: post-inflammatory phase

After 2–3 weeks, clinical examination of the skin revealed only a slight degree of residual erythema and scaling with no oedema or other changes. The cellular infiltrate had also substantially decreased compared to the Group II samples, and was approaching the baseline/group I values. Epidermal keratinocyte ICAM-1 was only focally present in three of our patients, and undetectable in the other four. However, the endothelial cell expression of ICAM-1, ELAM-1 and VCAM-1, while diminished, was still elevated over baseline values indicating persistent expression of both ELAM-1 (Fig. 7a) and

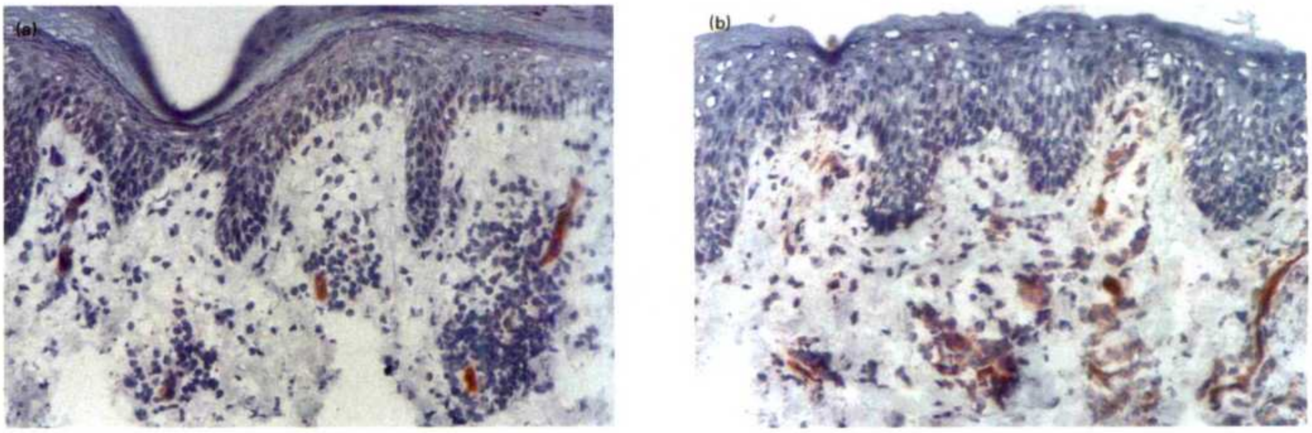

Figure 7. (a) ELAM-1 expression persisting on endothelial cells 3 weeks after treatment. (b) VCAM-1 expression persisting on endothelial cells 3 weeks after treatment.

VCAM-1 (Fig. 7b). The epidermal keratinocyte TNF-α and IL-8 staining pattern had largely reverted back to baseline levels.

Discussion

This experimental model was designed to detect *in vivo* the molecular mediators of adhesive interactions between leucocytes and cultured cytokine-activated endothelial cells with neutrophils, lymphocytes and determined where and when these molecules were expressed by indigenous and migrating cells that participate in adhesive interactions during the course of cutaneous inflammation. From tissue culture experiments it is known that at least three distinct mechanisms are involved for lymphocyte adhesion to endothelial cells.[15] The studies on the combination of human endothelial cells, with neutrophils, lymphocytes and monocytes have been reviewed.[16] In this present study we have assessed three distinct adhesion molecules, a T-cell chemotaxin (IL-8) and a modulatory cytokine (TNF-α), together with the associated accumulation of leucocytes in an *in-vivo* human system.[4,17]

Endothelial cell ELAM-1 was induced within 4–8 h following topical application of the poison ivy/oak mixture, and this induction preceded migration of lymphocytes into the dermis. ELAM-1 expression was not associated with an influx of neutrophils as predicted by *in vitro* experiments,[18] or as observed in baboon skin following intradermal injections of TNF-α and IFN-γ.[19] Moreover, *in vivo* ELAM-1 expression was more persistent (> 3 weeks) than previously observed for cultured endothelial cells[13] and endothelium in short-term skin organ cultures incubated with various cytokines,[20] in which ELAM-1 expression was essentially absent after only 24 h. The differences in results between cultured endothelial cells[13] and the current study may be partly explained by the experimental methods used. The study of cultured endothelial cells emphasized cell-surface immunoreactivity, whereas in frozen sections of intact skin specimens fixed with acetone, the resulting membrane permeabilization does not allow easy distinction between cytoplasmic and cell surface staining. It should be noted that different ELAM-1 antibodies have been used, and it is possible that the H4/18 antibody recognizes a different epitope on endothelial cells than the currently used BB11 antibody. However, it is also possible that the regulation of ELAM-1 expression and functional binding interactions between neutrophils and cultured endothelial cells is quite different from regulatory pathways *in vivo*. There may be pulses of endothelial cell activation *in vivo*, operating on a large reservoir of vascular-lining cells, which could give the appearance of persistence by individual cells, of ELAM-1 expression and a cell-to-cell contiguity.

Endothelial cell VCAM-1 was induced within 4–8 h following topical application of the poison ivy/oak mixture, and this induction preceded migration of lymphocytes into the dermis. This VCAM-1 expression occurred much earlier than that observed at 72 h in a delayed-type, cutaneous inflammatory reaction.[7] However, the rapid induction and prolonged expression of endothelial VCAM-1 is very similar to the results obtained by treating cultured endothelial cells with TNF-α.[14] The perivascular dermal dendritic cells were also VCAM-1 positive both in untreated control skin, and in inflamed skin which agrees with a previous study using a different anti-VCAM-1 antibody (INCAM-110).[7]

The induction of keratinocyte ICAM-1 occurred very rapidly before the development of a dermal T-lymphocytic infiltrate and an ICAM-1 network was formed in the upper portion of the skin involving endothelial cells, dermal dendritic cells, and basal keratinocytes.[2] At 2–3 weeks post-treatment when the cutaneous inflammation was resolving and there was no further trafficking of mononuclear cells into the epidermis, the keratinocytes became ICAM-1 negative. This temporal and spatial co-localization of keratinocyte ICAM-1 with intraepidermal lymphocyte trafficking[17] is found in several skin diseases (psoriasis, lichen planus, alopecia areata) before and during treatment with cyclosporin A.[21–23] We previously observed the ability of urushiol to activate directly keratinocyte ICAM-1 expression,[2] and noted that urushiol strongly induced TNF-α expression on cultured keratinocytes.[4] We therefore examined whether TNF-α protein expression could be detected early in our *in vivo* model, as this is the only known cytokine capable of inducing endothelial cell ELAM-1, VCAM-1 and keratinocyte ICAM-1 expression.

Keratinocyte TNF-α expression was induced within 4–8 h following topical application of the poison ivy/oak mixture, and the keratinocyte was the principal cutaneous cell type which was TNF-α positive. The ability of the keratinocytes to rapidly respond to urushiol by producing TNF-α, which could autoinduce keratinocyte ICAM-1 and then activate the underlying dermal endothelial cells, supports our earlier contention that the keratinocyte regulates the movement of dermal cells.[4,17,24] While the notion that the 'activated' endothelial cell is pivotal in T-cell-mediated inflammatory reactions has gained support in recent years,[1,11,25] the current results suggest that the epithelium, and perhaps perivascular dendritic macrophages, are very important in the endothelial cell–T-cell interactions, and may even initiate them.[4]

Keratinocyte IL-8 expression occurred somewhat later than the appearance of TNF-α, but correlated well with the migration of T lymphocytes towards the epidermis. This *in vivo* correlation involving keratinocyte TNF-α expression with the co-ordinated induction of ICAM-1 and IL-8, resembles that found *in vitro* and supports the notion that keratinocyte-derived IL-8 is an important T-cell chemotaxin both *in vitro*, and *in vivo*.[26]

In conclusion, these results indicate that the molecular basis for the precise cellular movement of leucocytes during the genesis, evolution, and termination of cutaneous inflammation induced by poison ivy/oak involves the production of TNF-α by epidermal keratinocytes, with the subsequent induction and diminution of multiple adhesion molecules on endothelial cells (ELAM-1, VCAM-1) and keratinocytes (ICAM-1), as well as the production of chemotactic factors such as IL-8. While there are almost certainly other components of this molecular and cellular network beyond those studied here, this experimental system can further our understanding of normal immunohomeostasis and the pathophysiology of acute and chronic cutaneous inflammation.

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References

1 Dvorak AM, Mihm MC Jr, Dvorak HF. Morphology of delayed type hypersensitivity reactions in man. II. Ultrastructural alterations affecting the microvasculature. *Lab Invest* 1976; **34:** 179–91.

2 Griffiths CEM, Nickoloff BJ. Keratinocyte intercellular adhesion molecule-1 (ICAM-1) expression precedes dermal T lymphocytic infiltration in allergic contact dermatitis (Rhus dermatitis). *Am J Pathol* 1989; **135:** 1045–53.

3 Barker JNWN, Sarma V, Mitra RS *et al.* Marked synergism between TNF-α and IFN-γ in regulation of keratinocyte-derived adhesion molecules and chemotactic factors. *J Clin Invest* 1990; **85:** 605–8.

4 Barker JNWN, Griffiths CEM, Dixit VM, Nickoloff BJ. Keratinocytes as initiators of inflammation. *Lancet* 1991; **337:** 211–14.

5 Carlos TM, Schwartz BR, Kovach NL *et al.* Vascular cell adhesion molecule-1 (VCAM-1) mediates lymphocyte adherence to cytokine-activated cultured human endothelial cells. *Blood* 1990; **76:** 965–70.

6 Cotran RS, Gimbrone MA, Bevilacqua MP *et al.* Induction and detection of a human endothelial activation antigen *in vivo*. *J Exp Med* 1986; **164:** 661–6.

7 Rice GE, Munro JM, Bevilacqua MP. Inducible cell adhesion molecule 110 (INCAM-110) is an endothelial receptor for lymphocytes. *J Exp Med* 1990; **171:** 1369–74.

8 Nickoloff BJ, Karabin GD, Barker JNWN *et al.* Molecular and cellular localization of IL-8 and its inducer—TNF-α in psoriasis. *Am J Pathol* 1991; **138:** 129–40.

9 Cerio R, Griffiths CEM, Cooper KD *et al.* Characterization of factor XIIIa positive dermal dendritic cells in normal and inflamed skin. *Br J Dermatol* 1989; **121:** 421–31.

10 Sontheimer RD. Perivascular dendritic macrophages as immunobiological constituents of the human dermal microvascular unit. *J Invest Dermatol* 1989; **93** (Suppl.): 96–101.

11 Pober JS. Cytokine-mediated activation of vascular endothelium: Physiology and pathology. *Am J Pathol* 1988; **133:** 426–33.

12 Griffiths CEM, Voorhees JJ, Nickoloff BJ. Characterization of intercellular adhesion molecule-1 and HLA-DR in normal and inflamed skin: Modulation by recombinant interferon-gamma and tumor necrosis factor. *J Am Acad Dermatol* 1989, **20**: 617–29.

13 Pober JS, Bevilacqu MP, Mendrick DL *et al.* Two distinct monokines, interleukin-1 and tumor necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. *J Immunol* 1986; **136**: 1680–7.

14 Osborn L, Hession C, Tizzard R *et al.* Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. *Cell* 1989; **59**: 1203–11.

15 Dustin ML, Springer TA. Lymphocyte function associated antigen-1 (LFA-1) interaction with intercellular adhesion molecule-1 (ICAM-1) is one of at least three mechanisms for lymphocyte adhesion to cultured endothelial cells. *J Cell Biol* 1988; **107**: 321–31.

16 Stoolman LM. Adhesion molecules controlling lymphocyte migration. *Cell* 1989; **56**: 907–10.

17 Nickoloff BJ, Griffiths CEM, Barker JNWN. The role of adhesion molecules, chemotactic factors, and cytokines in inflammatory and neoplastic skin disease—1900 update. *J Invest Dermatol* 1990; **94** (Suppl.): 151–7.

18 Bevilacqua MP, Pober JS, Mendrick DL *et al.* Identification of an inducible endothelial-leukocyte adhesion molecule. *Proc Natl Acad Sci USA* 1987; **84**: 9238–42.

19 Munro JM, Pober JS, Cotran RS. Tumor necrosis factor and interferon-γ induce distinct patterns of endothelial activation and associated leukocyte accumulation in skin of *Papio anubis*. *Am J Pathol* 1989; **135**: 121–33.

20 Messadi DV, Pober JS, Fiers W *et al.* Induction of an activation antigen on post-capillary venular endothelium in human skin organ culture. *J Immunol* 1987; **139**: 1557–62.

21 Ho VC, Griffiths CEM, Ellis CN *et al.* Intralesional cyclosporine A in the treatment of psoriasis: A clinical, immunologic and pharmacokinetic study. *J Am Acad Dermatol* 1990; **22**: 94–100.

22 Ho VC, Gupta AK, Ellis CN *et al.* Treatment of severe lichen planus with cyclosporin A. *J Am Acad Dermatol* 1990; **22**: 64–8.

23 Gupta AK, Ellis CN, Cooper KD *et al.* Oral cyclosporine A for the treatment of alopecia areata. *J Am Acad Dermatol* 1990; **22**: 242–50.

24 Nickoloff BJ. The role of gamma interferon in cutaneous trafficking of lymphocytes with emphasis on molecular and cellular adhesion events. *Arch Dermatol* 1988; **124**: 1835–43.

25 Willms-Kretschmer K, Flax MH, Cotran RS. The fine structure of the vascular response in hapten-specific delayed hypersensitivity and contact dermatitis. *Lab Invest* 1967; **17**: 334–9.

26 Larsen CG, Anderson AO, Oppenheim JJ, Matsushima K. Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumor necrosis factor. *Immunology* 1989; **68**: 31–6.

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Expression of a cell adhesion protein (VLA β) in normal and diseased skin

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Summary

Biopsies from normal skin ($n=17$) and various cutaneous disorders ($n=83$) were examined immunohistologically for reactivity with an antibody (CD29) against the common β chain of the VLA integrin family. In normal skin, CD29 recognized a number of cell types, i.e. endothelial cells, fibroblasts, T lymphocytes and basal keratinocytes. Similar cells were positive in diseased skin, but the expression of VLA β was upregulated on keratinocytes. The phenotype of the VLA β-positive T cells was examined in more detail by staining with anti-T-cell antibodies, i.e. CD3, CD4, CD8, CD45RO (UCHL1) and CD45R (2H4). These studies showed that most of the T cells in normal skin, benign cutaneous conditions and early cutaneous T-cell lymphomas (CTCL) expressed a similar phenotype and resembled antigen committed 'memory' (helper/inducer) cells ($CD4^+$, $CD29^+$, $CD45RO^+$, $CD45R^-$). In advanced CTCL, expression of these antigens was more variable, and many of these infiltrates showed aberrant (or unusual) expression of CD29, CD45RO, CD45R and other T-cell antigens. It is concluded that several cells involved in cutaneous immune reactions express a molecule (VLA β) which acts as a receptor for extracellular matrix components. This molecule is important for the attachment of cells to connective tissue constituents and may act to facilitate the migration of lymphocytes (and other cells) during immune reactions in normal and diseased cutaneous conditions. Advanced CTCL differ from the early lesions and it is possible that there is a progressive accumulation of increasingly malignant (or transformed) cells in these conditions.

Cell adhesion is critical for a number of immune reactions. Leucoyctes must attach to endothelial cells and to extracellular matrix components so that migration of leucocytes to sites of inflammation can occur. Leucocytes must also attach to antigen-presenting cells (or target cells) before specific immune reactions can occur.

Several molecules are implicated in these interactions, including a group of proteins collectively referred to as integrins, which are homologous in structure and consist of heterodimers composed of one α chain (unique to each individual molecule) and one β chain (common to different molecules within one family). Three families of integrins are recognized: the VLA integrins, the leucocyte integrins, and the cytoadhesion integrin family (see Table 1).[1–3]

Most previous studies of the skin have focused upon the leucocyte integrins. These studies have shown that endothelial cells, fibroblasts, lymphocytes and in some instances keratinocytes may express a molecule which acts as a ligand for lymphocyte function antigen-1 (LFA-1), intercellular adhesion molecule-1 (ICAM-1).[4–6] Knowledge of the expression of other cell adhesion proteins in cutaneous biopsies is more limited.

In this study, we have examined biopsy samples from normal skin and various cutaneous disorders for reactivity with an antibody (CD29) against the common β chain of the VLA integrin family. In all cases, the phenotype of the T-cell infiltrate was also examined by staining with anti-T-cell antibodies, i.e. CD3, CD4, CD8, CD45RO (UCHL1) and CD45R (2H4).

Methods

Biopsy samples

Biopsy samples were obtained from normal skin from 17 healthy adult volunteers and from 26 patients with benign cutaneous conditions: patch-test infiltrates (six cases), eczema (five cases), lichen planus (eight cases) and psoriasis (seven cases). Samples from 57 patients

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Table 1. Integrin molecules

Molecules*	Subunits	CD designations†	Known ligands‡
VLA integrins			
VLA-1	α_1/β_1	CDw49a/CD29	LA, Coll
VLA-2	α_2/β_1	CDw49b/CD29	Coll
VLA-3	α_3/β_1	CDw49c/CD29	LA, Coll, FN
VLA-4	α_4/β_1	CDw49d/CD29	FN
VLA-5	α_5/β_1	CDw49e/CD29	FN
VLA-6	α_6/β_1	CDw49f/CD29	LA
Leucocyte integrins			
LFA-1	α_L/β_2	CD11a/CD18	ICAM-1
Mac 1	α_M/β_2	CD11b/CD18	C3bi
p150,95	α_X/β_2	CD11c/CD18	C3bi
Cytoadhesion integrins			
VNR	α_V/β_3	CD51/CD61	VN
gpIIb/IIIa	α_{IIb}/β_3	CD41/CD61	VN, FN, FB, vWF

*VLA, very late activation; LFA-1, lymphocyte function antigen-1; VNR, vitronectin receptor, gp, glycoprotein.

†CD designations CDw49a, c and e are as yet not formally established, but seem likely.

‡LA, laminin; Coll, collagen; FN, fibronectin; ICAM-1, intercellular adhesion molecule-1; VN, vitronectin; FB, fibrinogen; vWF, von Willebrand factor.

with known or suspected cutaneous T-cell lymphomas (CTCL) were classified in accordance with standard criteria (Table 2).[7]

Processing and staining of biopsy samples

Fresh samples were frozen in a mixture of 2-methylbutane and dry ice and stored at −80°C. Cryostat sections were air-dried overnight at room temperature, fixed in acetone for 10 min and then stained with an IgG1 antibody (4B4) against the common β chain of the VLA integrin family.[8] In all cases, the phenotype of the T-cell infiltrate was further examined by staining with anti-T-cell antibodies, anti-Leu 4 (CD3), anti-Leu 3 (CD4), anti-Leu 2 (CD8), UCHL1 (CD45RO) and 2H4 (CD45R). Selected cases with unusual expression of these antigens were also stained with OKT6 (CD1), anti-Leu 5 (CD2), anti-Leu 2 (CD5), anti-Leu 9 (CD7), anti-Leu 8, βF1 (against a framework determinant on the T-cell receptor (TCR) β chain)[9] and TCRδ1 (against a framework determinant on the TCR δ chain).[10] The staining was performed using a three-stage immunoperoxidase method.[7]

Positive controls were performed by staining lymphoid organs with benign hyperplasia, and negative controls by omitting the primary antibodies.

Results

Normal skin (n = 17)

In normal skin, staining with 4B4 (CD29) labelled fibroblasts, nerves, endothelial cells and basal keratinocytes (Fig. 1a). Keratinocytes in the stratum spinosum and stratum granulosum were CD29-negative, and staining of Langerhans cells was not observed. The glandular and follicular epithelium was positive, but reacted more weakly than the basal keratinocytes. Basal membranes around blood vessels were strongly CD29-positive in all cases (see Fig. 1a). In contrast, no staining was seen of the basal membranes underneath the surface epithelium.

In all specimens, small numbers of T lymphocytes (positive for CD3) were present in the dermis and/or at the dermo-epidermal junction. The great majority of these cells were positive for CD29 and resembled antigen committed 'memory' (helper/inducer) cells in phenotype ($CD4^+$, $CD29^+$, $CD45RO^+$).

Staining with CD8 showed scattered positive cells and most of these were present at the dermo-epidermal junction. Staining with CD45R (2H4) was negative or confined to occasional cells in the epidermis and/or dermis.

Table 2. Expression of CD29 (4B4) and CD45R (2H4) in cutaneous T-cell lymphomas

Diagnosis	No. of cases	$CD29^+$, $CD45R^-$	$CD29^-$, $CD45R^+$	$CD29^+$, $CD45R^+$	$CD29^-$, $CD45R^-$
MF, suspected	18	18	0	0	0
MF, plaque stage	8	7	0	0	1
MF, tumour stage	12	6	1	4	1
Sézary syndrome	8	8	0	0	0
CTCL, large cells	11	7	2	1	1

MF, mycosis fungoides; CTCL, large cells, cutaneous T-cell lymphomas of pleomorphic ($n = 9$) or anaplastic ($n = 2$) subtype.

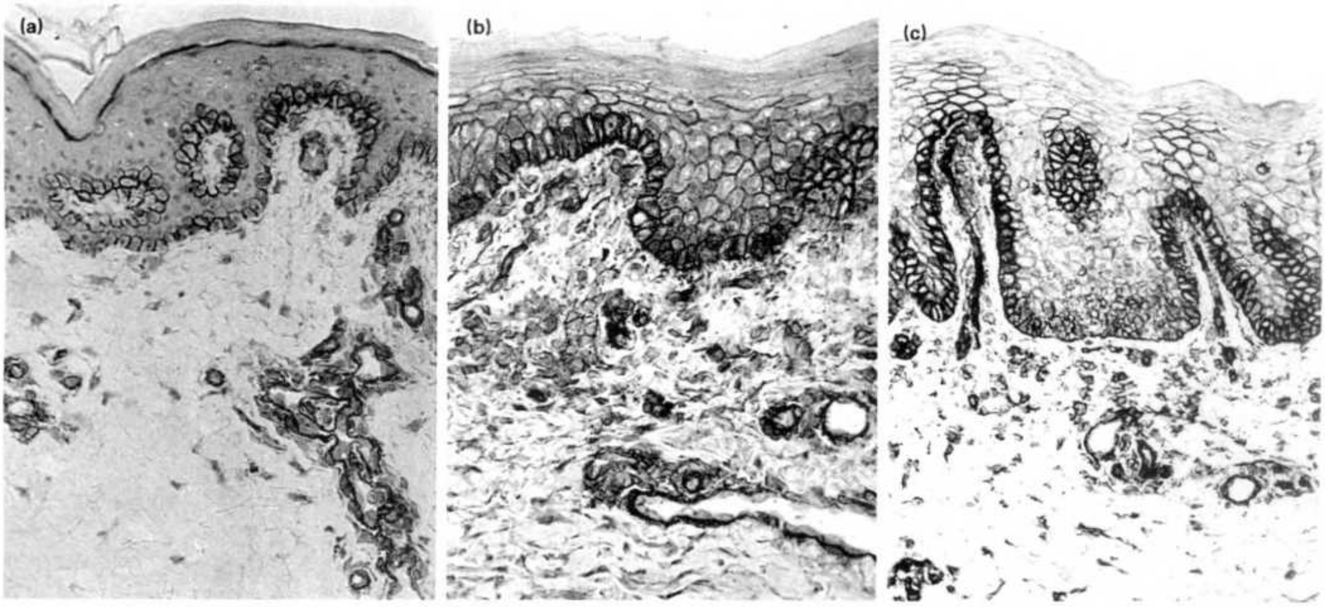

Figure 1. Biopsy samples from (a) normal skin (× 300), (b) lichen planus (× 400) and (c) early mycosis fungoides (× 400) stained for VLA β (CD29) with the use of a three-stage immunoperoxidase method. The reactivity pattern is similar in the three biopsies. In all cases, there is a labelling of lymphocytes, fibroblasts, endothelial cells and keratinocytes. In normal skin (a), staining of the keratinocytes is confined to the basal cells. In diseased skin (b and c), there is also labelling of superficial keratinocytes in the stratum spinosum. Note that the dark material seen in the stratum granulosum in the normal skin in (a) does not represent immunoreactive material, but results from the staining of cytoplasmic keratohyalin and pyknotic nuclei with haematoxylin.

Benign cutaneous disorders ($n = 26$)

Staining with 4B4 (CD29) in various benign inflammatory disorders showed a similar pattern of reactivity and differences between individual disorders were not observed. In all cases, CD29 reacted with fibroblasts, nerves and endothelial and adnexal cells. Keratinocytes were also CD29-positive, including not only the basal cells, but also superficial cells in the stratum spinosum (Fig. 1b).

All the benign cutaneous disorders studied showed dermal and epidermal infiltrates of CD3-positive T cells. The majority of these cells expressed a phenotype similar to that of T cells seen in normal skin, being positive for

Table 3. Phenotype of the neoplastic T cells in 11 patients in which unusual expression of CD29 and CD45R was seen

Case	CD1	CD2	CD3	CD4	CD5	CD7	CD8	CD29	CD45R	CD45RO	βF1	TCRδ1	Leu 8
Mycosis fungoides, plaque													
1.	−	−	+	+	+	−	−	−	−	+	−	−	+
Mycosis fungoides, tumour													
2.	−	−	+	+	+	−	−	−	−	−	+	−	−
3.	−	−	+	−	+	−	−	+	+	−	+	−	−
4.	−	−	+	−	+	+	+	+	+	+	+	−	−
5.	−	−	+	−	−	−	−	+	+	+	−	+	−
6.	−	−	+	+	+	+	−	−	+	+	−	−	+
7.	−	+	+	−	+	−	−	+	+	+	−	−	−
CTCL, pleomorphic or anaplastic													
8.	−	+	+	+	−	−	−	−	−	+	−	−	+
9.	−	−	+	+	+	+	−	+	+	−	+	−	−
10.	−	+	+	+	+	−	−	−	+	+	+	−	−
11.	−	−	+	−	+	+	+	−	+	+	−	−	−

CD4, CD29 and CD45RO. Many CD45R (2H4)-positive T cells were seen in one case of lichen planus and one of eczema. The biopsy samples from the remaining 24 benign cutaneous disorders contained none or only very few CD45R (2H4)-positive cells. A predominance of CD8-positive cells was not seen in any of the cases.

Cutaneous T-cell lymphomas (n = 57)

In cutaneous T-cell lymphomas (CTCL), staining of keratinocytes, fibroblasts, nerves and endothelial and adnexal cells was similar to that seen in the benign cutaneous disorders described above (Fig. 1c).

The results from the labelling of the infiltrating T cells are summarized in Table 2. In suspected CTCL, the phenotype of the T-cell infiltrate was similar to that seen in normal skin and benign cutaneous conditions (CD4$^+$, CD29$^+$, CD45RO$^+$). In these cases, staining with CD8 and CD45R (2H4) was negative or confined to occasional cells. Similar findings were seen in Sézary syndrome and in all but one of the plaque lesions of mycosis fungoides (MF). The T cells in the remaining plaque

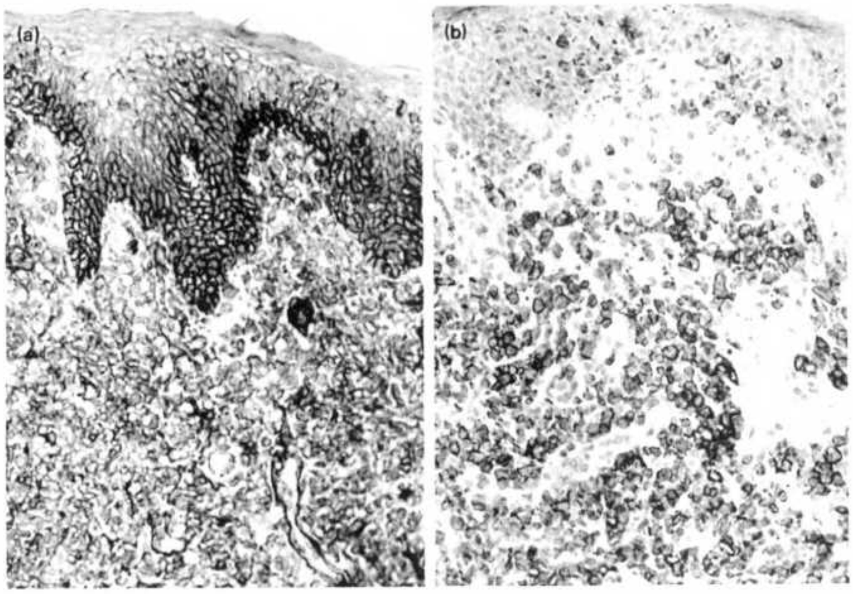

Figure 2. A cutaneous T-cell lymphoma of pleomorphic subtype stained with (a) CD29 (4B4 ×400) and (b) CD45R (2H4 ×400). The neoplastic cells are positive for both molecules.

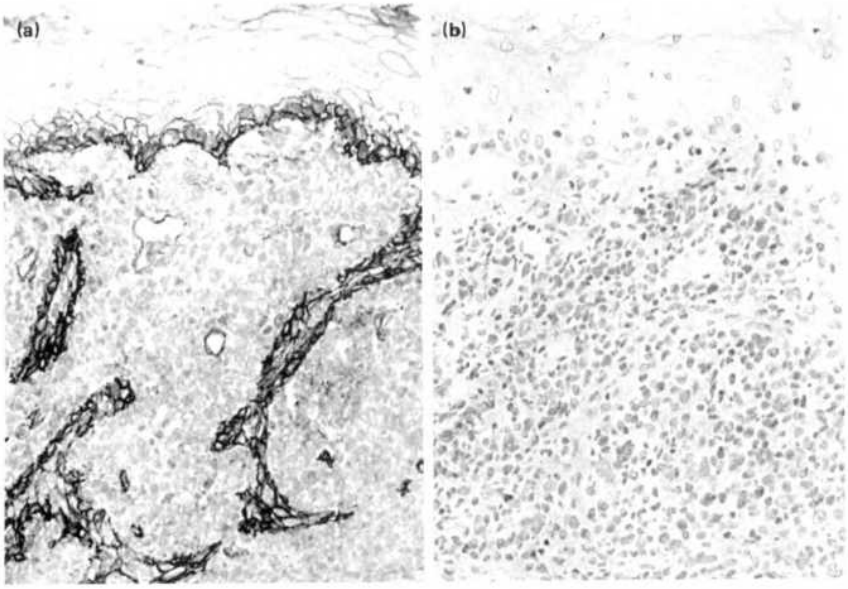

Figure 3. A cutaneous T-cell lymphoma of anaplastic subtype stained with (a) CD29 (4B4 ×400) and (b) CD45R (2H4 ×400). The neoplastic cells are negative for both molecules.

lesion were negative for both CD29 and CD45R, and the phenotype of these cells is summarized in Table 3.

In the tumour lesions of MF ($n = 12$) and the large cell lymphomas of pleomorphic or anaplastic subtype ($n = 11$), staining with CD29 (and CD45R) was much more variable. The neoplastic cells in 10 of these cases were either CD29$^-$ CD45R$^+$ or negative (or positive) for both molecules (Table 2, Figs 2 and 3). Examination of these infiltrates with a more extensive panel of anti-T-cell antibodies showed that most lymphomas with unusual expression of CD29 and CD45R had multiple immunophenotypic abnormalities (Table 3).

Discussion

The VLA antigens were first identified on *in-vitro* activated T lymphocytes and were so named because they appear very late after activation of T lymphocytes with lectin and/or antigen.[11] Subsequent studies helped to elucidate the molecular nature of these antigens, and it is now clear that the VLA antigens are integrin molecules and that several of them act as receptors for extracellular matrix components.[1-3]

Most previous studies on the expression of VLA β (CD29) have focused on isolated blood lymphocytes. These *in-vitro* studies have shown that two populations of CD4-positive T cells may be disintinguished depending upon the presence or absence of CD29. The CD29-positive CD4 cells are negative for CD45R (2H4) and positive for CD45RO (UCHL1) and act *in vitro* as antigen committed 'memory' (helper/inducer) cells.[8,12] Circulating CD4 cells with the reciprocal phenotype (CD29$^-$, CD45R$^+$, CD45RO$^-$) behave as virgin (or naive) cells and can induce suppression in immune reactions.[12] Knowledge of the expression of VLA β in intact tissues such as biopsy samples, is more limited.

In this study, we have examined biopsies from normal skin and various cutaneous disorders for reactivity with an antibody (CD29) against the common β chain of the VLA integrin family. The results confirm and extend other recent reports and indicate that CD29 recognizes a number of cell types in normal skin: fibroblasts, endothelial cells, T lymphocytes and basal keratinocytes.[13,14] In our study, labelling of Langerhans cells was not observed. This is in keeping with another light microscopic study of normal skin,[13] but contrasts with the report of Staquet *et al.* in which VLA β was identified on Langerhans cells by immunoelectron microscopy.[14] The reason for this discrepancy is not clear but it may relate to the sensitivity of the detection assay. Thus Staquet *et al.*[14] reported that the density of VLA β is much lower on Langerhans cells than on other cell types. It is possible that a small number of VLA β molecules may not be detectable by immunohistological examination at a light microscopic level.

The expression of VLA β in benign cutaneous disorders has been studied by Bos *et al.*[15] These authors focused upon the infiltrating T cells and reported that the T cells in specimens from 23 patients expressed a similar phenotype and were positive for CD4 and CD29 and negative for CD45R (2H4). The similar findings in this study support the view that most benign cutaneous T-cell infiltrates show a predominance of T cells which resemble antigen-committed 'memory' (helper/inducer) cells in phenotype. In this study, occasional benign cutaneous T-cell infiltrates contained many CD45R(2H4)-positive suppressor/inducer-like cells. This contrasts with the earlier report, but may not be surprising in view of the fact that interactions between inducers of help (CD29$^+$) and suppression (CD45R$^+$) are required during normal immune reactions.[12]

In the study of Bos *et al.*[15] no data were given as to the expression of VLA β by non-lymphoid cells. Our results indicate that there is an upregulated expression of VLA β on keratinocytes in inflammatory conditions as compared with keratinocytes in normal skin. The implication of this finding is not clear. However, it does suggest that VLA β may be important for the migration of keratinocytes during tissue repair and remodelling in diseased conditions.

Knowledge concerning the expression of VLA β (and other antigens on immunoregulatory T-cell subsets) in CTCL is limited. Sterry and Mielke studied specimens from 23 patients and reported that the neoplastic T cells resembled antigen-committed memory cells (CD4$^+$, CD29$^+$, CD45RO$^+$, CD45R$^-$) in all cases.[16] In their study aberrant expression of T-cell antigens was not identified. In another report of 15 patients, two cases were seen in which the neoplastic T cells were positive for both CD45RO and CD45R.[17] The present study confirms and extends this latter report and indicates that aberrant (or unusual) expression of CD29 and CD45R is not uncommon in CTCL. Some cases are dissimilar from known, normal T-cell subsets and are negative (or positive) for both molecules. Others are unusual in that they do not resemble memory T cells, but express the reciprocal phenotype (CD29$^-$, CD45$^+$) normally seen on naive T cells.[12]

In this study these aberrant (or unusual) T-cell phenotypes were seen almost exclusively in advanced CTCL. By contrast, in the early lesions the expression of CD29 and CD45R (and other T-cell antigens) was similar to that seen in benign disorders. Taken together, these findings support the view that fundamental differences exist between early and advanced CTCL. It is conceivable that there is a progressive accumulation of increasingly malignant (or transformed) cells in the advanced stages, and this may account for the greater frequency of aberrant (or unusual) expression of T-cell antigens, such as for example VLA β, in these conditions. Whether differences also exist with respect to the expression of VLA α chains between the T cells in benign cutaneous T-cell infiltrates and early and advanced CTCL is not yet known.

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References

1 Hynes RO. Integrins: a family of cell surface receptors. *Cell* 1987, **48**: 549–54.

2 Hemler ME. Adhesive protein receptors on hematopoietic cells. *Immunology Today* 1988; **9**: 109–13.

3 Kishimoto TK, Larson RS, Corbi AL *et al.* Leukocyte integrins. In: *Leukocyte Adhesion Molecules* (Springer TA, Anderson DC, Rosenthal AS, Rothlein R, eds), Springer-Verlag, 1990; 7–43.

4 Dustin RL, Rothlein R, Bhan AK *et al.* Induction by IL-1 and inteferon-γ. Tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1) *J Immunol* 1986; **137**: 245–54.

5 Griffiths CEM, Voorhees JJ, Nickoloff BJ. Characterization of intercellular adhesion molecule-1 and HLA-DR expression in normal and inflamed skin: modulation by recombinant gamma interferon and tumor necrosis factor. *J Am Acad Dermatol* 1989; **20**: 617–29.

6 Vejlsgaard GL, Ralfkiaer E, Avnstorp C *et al.* Kinetics and characterisation of intercellular adhesion molecule-1 (ICAM-1) expression on keratinocytes in various inflammatory skin lesions and malignant cutaneous lymphomas. *J Am Acad Dermatol* 1989; **20**: 782–90.

7 Ralfkiaer E, Wantzin GL, Mason DY *et al.* Phenotypic characterization of lymphocyte subsets in mycosis fungoides: comparison with large plaque parapsoriasis and benign chronic dermatoses. *Am J Clin Pathol* 1985; **84**: 610–9.

8 Morimoto C, Letvin NL, Boyd AW *et al.* The isolation and characterization of the human helper inducer T cell subset. *J Immunol* 1985; **134**: 3762–9.

9 Brenner MB, McLean J, Scheft H *et al.* Characterization and expression of the human alpha/beta T-cell receptor by using a framework monoclonal antibody. *J Immunol* 1987; **138**: 1502–9.

10 Faure F, Jitsukawa S, Triebel F, Hercend T. Characterization of human peripheral lymphocytes expressing the CD3-gamma/delta complex with anti-receptor monoclonal antibody. *J Immunol* 1988; **141**: 3357–60.

11 Hemler ME, Huang C, Schwarz L. The VLA protein family. Characterization of five distinct cell surface heterodimers each with a common 130,000 molecular weight β subunit. *J Biol Chem* 1987; **262**: 3300–9.

12 Sanders ME, Makgoba MW, Shaw S. Human naive and memory T cells: reinterpretation of helper–inducer and suppressor–inducer subsets. *Immunol Today* 1988; **9**: 195–9.

13 Konter U, Kellner I, Klein E *et al* Adhesion molecule mapping in normal human skin. *Arch Dermatol Res* 1989; **281**: 454–62.

14 Staquet MJ, Dezutter-Dambuyant C, Schmitt D *et al.* A surface glycoprotein complex related to the adhesive receptors of the VLA family shared by epidermal Langerhans cells and basal keratinocytes. *J Invest Dermatol* 1989; **92**: 739–45.

15 Bos JD, Hagenaars C, Das PK *et al* Predominance of 'memory' T cells ($CD4^+$, $CDw29^+$) over 'naive' T cells ($CD4^+$, $CD45R^+$) in both normal and diseased human skin. *Arch Dermatol Res* 1989; **281**: 24–30.

16 Sterry W, Mielke V. $CD4^+$ cutaneous T-cell lymphomas show the phenotype of helper/inducer cells ($CD45RA^-$, $CDw29^+$). *J Invest Dermatol* 1989; **93**: 413–6.

17 Nickoloff BJ, Griffiths CEM. Intraepidermal but not dermal T lymphocytes are positive for a cell-cycle-associated antigen (Ki-67) in mycosis fungoides. *Am J Pathol* 1990; **136**: 261–6.

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Immunoelectron microscopic characterization of a subpopulation of freshly isolated epidermal Langerhans cells that reacts with anti-CD23 monoclonal antibody

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Summary Large subsets of leucocytes were recently shown to express the low affinity receptor for the Fc portion of IgE. Because Langerhans cells (LC) are epidermal leucocytes, we investigated whether LC of normal human subjects might express this receptor. Whereas conventional immunofluorescence on epidermal sheets gave negative results, highly sensitive immunoelectron microscopy revealed that a subset (about one-third) of freshly isolated LC express the CD23 molecule.

The low affinity receptor for the Fc portion of immunoglobulin (Fc-Ig) E is a single-chain glycoprotein that consists of 321 amino acids, orientated with its amino-terminus on the cytoplasmic side[1] and its carboxy-terminus outside the cell.[1] In contrast to other Fc-Ig receptors, it has not evolved as a member of the immunoglobulin supergene family, but has substantial homology with several animal lectins.[1]

Human epidermal Langerhans cells (LC) express various membrane receptors. Using rosette assays LC were shown to bear the Fc-IgG receptor and the C3 receptor.[2] Recent investigations with monoclonal antibodies (MAb) have clarified the type of receptor present at the LC surface and have demonstrated that LC bear the 40-kDa Fc receptor for mouse IgG1,[3] as well as the C3bi receptor.[4,5]

Subsets of normal human peripheral blood leucocytes, including monocytes, T and B lymphocytes, platelets, eosinophils and natural killer (NK) cells, express the low affinity Fc receptor for IgE: Fc$_\epsilon$RII/CD23.[6] Because LC, which are known to belong to the monocyte–macrophage lineage, are considered epidermal leucocytes,[7,8] we investigated whether this receptor was present at the surface of LC of normal human epidermis using immunostaining procedures, including a pre-embedding immunoelectron microscopy (IEM) method, using two anti-Fc$_\epsilon$RII/CD23 MAb.[9]

Methods

Normal human skin specimens were obtained from five healthy volunteers, age range 23–58 years, and removed from either the breast or abdomen.

Reagents

The MHM6 (IgG1) MAb (Dakopatts, Glostrup, Denmark) and the HD50 (IgG2b) MAb (Oxford Workshop on leucocyte differentiation antigens, 1986; provided by Dr Soligo, Milan) were used as anti-CD23 MAb. The following secondary antibodies were used: fluorescein isothiocyanate-conjugated F(ab')$_2$ goat anti-mouse IgG antibody (Tago, Inc., Burlingame, CA, U.S.A.) for immunofluorescence (IF) and peroxidase-conjugated-F(ab')$_2$ sheep anti-mouse Ig antibody (Amersham, Buckinghamshire, U.K.) for immunoperoxidase (IP) IEM labelling. Control immunoreagents included purified mouse unreactive Ig fractions of the isotypes IgG1 and IgG2b (Chemicon International Inc., El Segundo, CA, U.S.A.) and purified anti-CD1a MAb (Becton-Dickinson Monoclonal Center, Mountain View, CA, U.S.A.).

Immunofluorescence studies on epidermal sheets[4]

Fresh biopsy samples were trimmed and floated on a 0·25% solution of trypsin (Gibco, Paisley, U.K.) in Hanks' balanced salt solution (HBSS, Gibco) for 45 min

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at 37°C. The epidermal sheets, after being washed in phosphate-buffered saline (PBS), were incubated for indirect immunostaining with (first layer) the anti-CD23 MAb (or the control anti-CD1a MAb on separate sheets) at varying dilutions in PBS for 6 h at 4°C, followed by (second layer) the fluorescein isothiocyanate-conjugated secondary antibody diluted 1:10 in PBS for 12 h at 4°C. After washes in PBS and mounting in PBS/glycerol, the sheets were examined under a Zeiss fluorescence microscope.

Epidermal cell suspension and Langerhans cell enrichment

The skin specimens were sliced with a keratotome and these slices were floated, dermal side down, on ammonium thiocyanate solution as previously described.[10] The resulting epidermal sheets were treated with 0·25% trypsin in HBSS for 10 min at 37°C, shaken and pipetted in HBSS supplemented with 10% heat-inactivated fetal calf serum (FCS, Gibco). The resulting suspended cells were sedimented by centrifugation, resuspended and washed in RPMI-1640 medium with 10% FCS (RPMI-FCS).

For LC enrichment, epidermal cells (EC) dispersed in 20 ml RPMI-FCS (5×10^6 cells/ml) were layered on 10 ml of the Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) gradient, and subsequently centrifuged at 1000 *g* for 45 min, collected and resuspended in RPMI-FCS. The percentage of LC enrichment was approximately 15%. The viability, assessed by eosin exclusion, was higher than 90%.

Immunoelectron microscopy studies on suspended cells

LC-enriched EC, after prefixation with 0·1% glutaraldehyde in 0·1 M sodium cacodylate buffer for 10 min, and quenching of aldehyde groups by 50 mM glycine, were subjected to a blocking pretreatment, including PBS with 1% bovine serum albumin (BSA, Sigma, St Louis, MO, U.S.A.), 0·2% sodium azide and 10% decomplemented human AB serum, for 30 min at 4°C. After washings in PBS-0·1% BSA, the anti-CD23 MAb were applied to specimen cells at varying dilutions in PBS-1% BSA for 30 min at 4°C. After washes in PBS-0·1% BSA, the peroxidase-conjugated secondary antibody was used, diluted 1:10 in PBS-1% BSA, for 60 min. After several washings in PBS-0·1% BSA, the cells were incubated with 3,3′ diaminobenzidine diluted 0·5–1 mg/ml in Tris-HCl buffer containing 0·01% hydrogen peroxide, for 5 min. After washing in Tris-HCl buffer, the cells were finally fixed with 1% glutaraldehyde in 0·1 M cacodylate buffer, subsequently packaged in Agar (Difco Laboratories, Detroit, MI, U.S.A.), postfixed with osmium tetroxide reduced with potassium ferricyanide, dehydrated, embedded, ultracut and examined according to standard ultrastructural procedures. Fifty LC were considered per sample, and the number of peroxidase-positive LC was scored.

Controls

For both IF on epidermal sheets and IP labelling in IEM, the negative controls consisted of replacement of the two MAb by mouse unreactive Ig fractions of the same isotypes (IgG1 and IgG2b) and replacement of the MAb by buffer solution. A positive control for immunofluorescence was the use of the anti-CD1a MAb instead of anti-CD23 MAb on the control epidermal sheets. As a final control the peripheral blood mononuclear cells (PBMC) were exposed to the same treatment, to determine whether the ammonium thiocyanate and the slight (10 min) trypsin exposure would alter the percentage of positive cells and the intensity of staining.

Results

No dendritic CD23-positive EC were visible using IF although some weak staining could occasionally be observed. By contrast, the LC in the control sheets were CD1a-positive.[4]

Under the electron microscope, epidermal cells showing the ultrastructural patterns of LC, including Birbeck granules, were considered CD23-positive if peroxidase deposits were detectable on the cell surface of positive cells (Fig. 1) especially at high magnification (Fig. 1b), and 31·2 ± 6·89 LC were positive. Keratinocytes (Fig.1) and melanocytes were negative for CD23. No peroxidase deposits were observed on the surface of control LC (Fig. 2), and slightly trypsinized PBMC consistently gave results similar to those obtained on untrypsinized PBMC.

Discussion

This study is the first to clearly demonstrate that a subset of LC, freshly isolated from normal human epidermis, expresses the antigen CD23. Unstimulated resting LC were recently shown to be negative for M-L25 and 3–5 (CD23), as assessed by indirect IF and rosette assay;[11] in other experiments specific rosette formation was seen only occasionally.[12] Similarly, IF staining of epidermal sheets in the present study gave apparently negative results. However, a subset of LC was shown to be CD23-

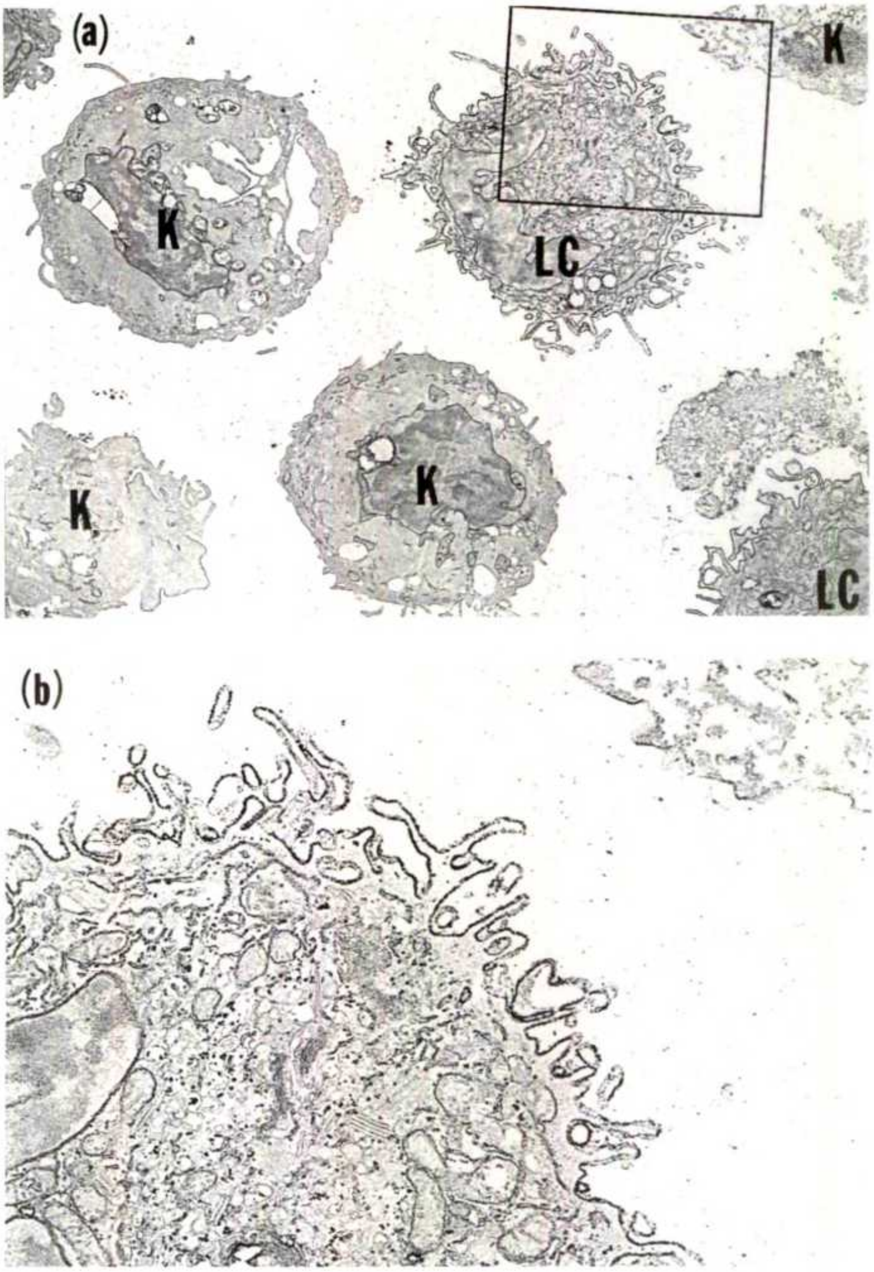

Figure 1 (a) Suspension of epidermal cells, including two CD23-positive (peroxidase-labelled) Langerhans cells (LC) and some CD23-negative (peroxidase unlabelled) keratinocytes (K) (× 8000). (b) Higher magnification of the area boxed in (a). Peroxidase deposits are visible on the surface of the CD23-positive Langerhans cell, but are lacking on the surface of the adjacent CD23-negative keratinocyte (× 23,000).

positive using IEM (Fig. 1), presumably due to the higher resolution provided by this ultrastructural technique.

The expression of CD23 on LC may not be fortuitous, since spatial and functional associations have been demonstrated on the membrane of leucocytes between CD23 and other molecules known to be expressed by resting human LC. CD23 was thought to be spatially associated with CD1a antigen,[12] and was found to be associated with HLA-DR antigen on B cells.[13] It is well established that CD1a and HLA-DR are two of the most characteristic markers of LC.[14] Also, CDw40 a surface protein of 50 kDa which is also present on the surface of a subset of resting human LC,[15] was found to be associated with CD23 on lymphocytes and macrophages.[16] In addition, CD23 was shown in functional assays to act in synergy with the C3bi receptor,[17] which is expressed on the surface of normal resting LC.[4,5]

It was postulated that CD23 plays an important role in antigen presentation because of the steric relationship between CD23 and HLA-DR.[13] A function for CD23 has

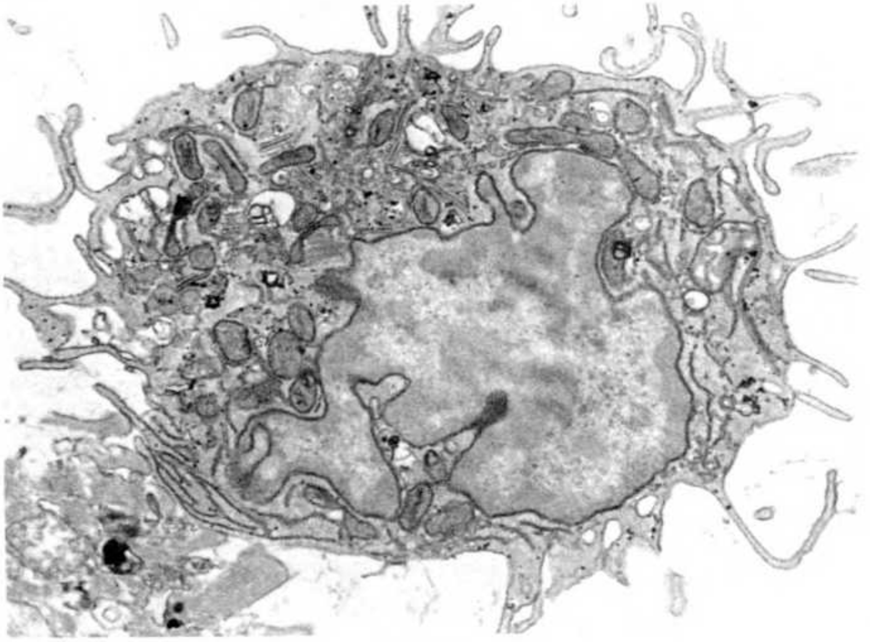

Figure 2. Negative control. A mouse unreactive fraction, of the same isotype, was used instead of the anti-CD23 MAb. Note that labelling is completely absent on the cell membrane of this control Langerhans cell (× 15,000).

also been shown in the mixed lymphocyte reaction (MLR) and in the stimulation of T cells by specific antigens presented on EBV-transformed B cells,[18] and soluble CD23 preparations can be co-stimulatory with certain mitogens for mature T cells.[18,19] Further, LC from atopic patients can present *in-vitro* inhalant antigens to T lymphocytes, and IgE molecules on LC were shown to be involved in the presentation of this antigen.[20] LC are well known to have antigen-presenting capacity (APC),[21,22] and CD23, expressed by a subset of LC, may have a role as a cofactor in the APC function, even in normal resting epidermis.

CD23 may also be involved in the adhesive functions of LC. The Arg-Gly-Asp (RGD) triplet of amino acids[1] is common to a number of molecules (i.e. fibronectin, laminin, vitronectin, collagen) that bind to receptors expressed by members of the integrin family of receptors, some of which are involved in cell–cell adhesion reactions.[18,23] In CD23 the RGD motif is located at the extracellular end and well outside the three compact heads formed from three cysteine pairs within the lectin homology region; this suggests that it could be available for binding to receptors on adjacent cells.[18] Such an interaction accounts for example, for the close association between the integrin gpIIb/IIIa and the CD23 expressed on platelets.[18,24] We may therefore speculate that the normal resting LC subset expressing CD23 may interact, through this molecule, with cells expressing on their surface glycoproteins of the integrin family of receptors, such as keratinocytes[25,26] and mononuclear leucocytes.[27]

Acknowledgments

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References

1 Kikutani H, Inui S, Sato R *et al.* Molecular structure of human lymphocyte receptor for Immunoglobulin E. *Cell* 1986; **47:** 657–65.

2 Stingl G, Wolff-Schreiner EC, Pichler WJ *et al.* Epidermal Langerhans cells bear Fc and C3 receptors. *Nature* 1977; **268:** 245–6.

3 Schmitt DA, Hanau D, Bieber T *et al.* Human Langerhans cells express only the 40 kD Fc receptor (FcγRII). *J Invest Dermatol* 1989; **92:** 514 A.

4 De Panfilis G, Soligo D, Manara GC *et al.* Adhesion molecules on the plasma membrane of epidermal cells. I. Human resting Langerhans cells express two members of the adherence-promoting CD11/CD18 family, namely, H-Mac-1 (CD11b/CD18) and gp 150,95 (CD11c/CD18). *J Invest Dermatol* 1989; **93:** 60–9.

5 De Panfilis G, Soligo D, Manara GC *et al.* Human normal-resting Langerhans cells do express the type 3 complement receptor. *Br J Dermatol* 1990; **122:** 127–36.

6 Delespesse G, Hofstetter H, Sarfati M *et al.* Human Fc_εRII. Molecular, biological and clinical aspects. In: *Structures and Functions of Low Affinity Fc Receptors* (Fridman WH, ed). *Chem Immunol* Vol. 47. Basel: Karger, 1989; 79–105.

7 Flotte TJ, Murphy GF, Bhan AK. Demonstration of T200 on human Langerhans cell surface membranes. *J Invest Dermatol* 1984; **82:** 535–7.

8 Romani N, Schuler G, Fritsch P. Ontogeny of Ia-positive and Thy-

1-positive leukocytes of murine epidermis. *J Invest Dermatol* 1986; **86**: 129–33.

9 Bonnefoy JY, Aubry JP, Peronne C *et al.* Production and characterization of a monoclonal antibody specific for the human lymphocyte low affinity receptor for IgE: CD23 is a low affinity receptor for IgE. *J Immunol* 1987; **138**: 2970–8

10 De Panfilis G, Manara GC, Ferrari C, Torresani C. Adhesion molecules on the plasma membrane of epidermal cells II. The intercellular adhesion molecule-1 (ICAM-1) is constitutively present on the cell surface of human resting Langerhans cells. *J Invest Dermatol* 1990; **94**: 317–21.

11 Bieber T, Rieger A, Neuchrist C *et al.* Induction of Fc$_\epsilon$R2/CD23 on human epidermal Langerhans cells by human recombinant interleukin 4 and γ interferon. *J Exp Med* 1989; **170**: 309–14.

12 Bruynzeel-Koomen C, Van Der Donk EMM, Bruynzeel PLB *et al.* Associated expression of CD1 antigen and Fc receptor for IgE on epidermal Langerhans cells from patients with atopic dermatitis. *Clin Exp Immunol* 1988; **74**: 137–42.

13 Bonnefoy JY, Guillot D, Spits H *et al.* The low-affinity receptor for IgE (CD23) on B lymphocytes is spatially associated with HLA-DR antigens. *J Exp Med* 1988; **167**: 57–72.

14 De Panfilis G. ATPase, antigens detected by monoclonal antibodies, ultrastructural characters: which is the best marker for Langerhans cells? *Arch Dermatol Res* 1983; **275**: 407–11.

15 Torresani C, Manara GC, Ferrari C *et al.* Demonstration of CDw40 antigen on the plasma membrane of Langerhans cells of normal human skin. *It Gen Rev Dermatol* 1989; **26**: 161–8.

16 Melewicz FM, Plummer JM, Spielgeberg HL. Comparison of the Fc receptors for IgE on human lymphocytes and monocytes. *J Immunol* 1982; **129**: 563–9.

17 Capron M, Kazatchkine MD, Fischer E *et al.* Functional role of the alpha-chain of complement receptor type 3 in human eosinophil-dependent antibody-mediated cytotoxicity against schistosomes. *J Immunol* 1987; **139**: 2059–65.

18 Gordon J, Flores-Romo L, Cairns JA *et al.* CD23: a multi-functional receptor/lymphokine? *Immunol Today* 1989; **10**: 153–7.

19 Armitage RJ, Goff LK, Beverley PCL. Expression and functional role of CD23 on T-cells. *Eur J Immunol* 1989; **19**: 31–6.

20 Bruynzeel-Koomen C, Boland G, Mudde GC. IgE-mediated antigens presentation by Langerhans cells from patients with atopic dermatitis. In: *The Langerhans Cell* (Thivolet J, Schmitt, D, eds), London: Colloque Inserm/John Libbey Eurotext, 1988; 335–41.

21 Stingl G, Katz SI, Clement L *et al.* Immunologic functions of Ia bearing epidermal Langerhans cells. *J Immunol* 1978; **121**: 2005–13.

22 Braathen LR, Thorsby E. Studies on human epidermal Langerhans cells. I Alloactivating and antigen presenting capacity. *Scand J Immunol* 1980; **11**: 401–8.

23 Hynes RO. Integrins: a family of cell surface receptors. *Cell* 1987; **48**: 549–54.

24 Capron A, Dessaint JP, Capron M *et al.* From parasites to allergy. a second receptor for IgE. *Immunol Today* 1986, **7**: 15–8.

25 Staquet MJ, Dezutter-Dambuyant C, Schmitt D *et al.* A surface glycoproteins complex related to the adhesive receptors of VLA family, shared by epidermal Langerhans cells and basal keratinocytes. *J Invest Dermatol* 1989; **92**: 739–45.

26 Zambruno G, Girolomoni G, Staquet MJ *et al.* CDw29 expression on Langerhans cells and on basal keratinocytes. In: *The Langerhans Cell* (Thivolet J, Schmitt D, eds). London: Colloque Inserm/John Libbey Eurotext, 1988; 159–66.

27 Sanchez-Madrid F, Nagy J, Robbins E *et al.* A human leukocyte differentiation antigen family with distinct α subunits and a common β subunit: the lymphocyte function-associated antigen (LFA-1), the C3bi complement receptor (OKM1/Mac-1), and the p 150,95 molecule *J Exp Med* 1983; **158**: 1785–803.

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Interleukin-1β stimulates fibroblast elastase activity

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Summary We report the stimulation of elastase activity of human skin fibroblasts by interleukin-1β (IL-1). Elastase activity was determined by the hydrolysis of the synthetic substrate succinoyl trialanine paranitroanilide (Suc(Ala$_3$)NA). Recombinant human IL-1β induced a concentration-dependent increase in the elastase activity of fibroblasts. The supernatants of keratinocyte cultures that contained IL-1/ETAF showed similar stimulatory activity.

Interleukin-1 (IL-1) is a widely found cytokine that is involved in various inflammatory, metabolic and immunological reactions.[1] The action of IL-1 on fibroblasts results in the stimulation of their growth[2] and enhancement of collagenase production[3,4] and inhibition of fibronectin synthesis.[5] The keratinocytes produce large amounts of IL-1/ETAF[6,7] and the epidermis is a major site for the biosynthesis of IL-1 in humans.[8] Thus IL-1 is an important mediator of keratinocyte–fibroblast interactions in the skin. We report on a new property of IL-1 on human skin—the stimulation of the elastase activity of fibroblasts.

Methods

Fibroblast cultures

Skin was obtained from three individuals aged 20–40 years undergoing plastic surgery. The dermal fibroblasts were cultured using an explant technique and cells between the 5th and 10th passage were plated at a concentration of 4×10^4 cells/cm^2 in 60-mm-diameter Petri dishes and cultured in a CO_2 incubator at 37°C using Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY, U.S.A.) supplemented with 5% fetal calf serum and 100 U/ml penicillin and 100 μg/ml streptomycin.[9] When the monolayer reached 90% confluence (1×10^6 cells) the culture medium was removed and the cells were then washed. Fresh culture medium was added with or without recombinant human cytokines or keratinocyte-conditioned medium. After incubation for 4, 24, 48, 72 and 96 h the supernatants were removed and the cell layers washed twice with 0·1 M Tris HCl buffer (pH 7·3) and the cells then scraped off from the plate and resuspended in buffer. They were either used immediately or stored at −20°C. The number of fibroblasts in parallel cultures was recorded using a Coulter counter.

Epidermal cell cultures and supernatants

Epidermal cell cultures were grown in 75-cm^2 culture flasks. PAM 212 cells were cultured using Dulbecco's modified Eagle's medium and normal human keratinocytes were grown to confluence as previously described.[10] The culture medium was replaced and the cultures incubated for an additional 48 h. The epidermal cell culture supernatant removed after this 48 h period is referred to as 'conditioned medium'.

Enzyme assays

The cells were disrupted by ultrasound and were then centrifuged at 5000 *g* for 10 min and the supernatant collected for determination of elastase activity. This was measured according to the method of Bieth *et al.*[11] using the synthetic elastase substrate succinoyl trialanine paranitroanilide (Suc(Ala$_3$)NA). Previous studies have shown that partially purified elastase from dermal fibroblasts is able to hydrolyse the substrate and exhibit elastolytic activity for human skin elastic fibres.[12] Briefly, aliquots of cell extracts were added to the reaction medium containing Tris buffer at pH 8·3 with 5 mM $CaCl_2$, 0·02% NaN_3 and 0·1% Brij. The reaction was started by the addition of a 100 mM stock solution of Suc(Ala$_3$)NA dissolved in N-methyl-2-pyrrolidone to

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give a final concentration of 1 mM. The enzyme–substrate mixture was incubated for 1 h in a 25°C water bath. The enzymatic reaction was stopped by the addition of glacial acetic acid. The release of paranitroanilide was recorded with time. The specific elastase activity was expressed as IU, i.e. nmoles of substrate hydrolysed/min/mg of protein. The protein content was determined according to the method of Lowry.

Human recombinant cytokines

Human recombinant IL-1β (specific activity $2{\cdot}5 \times 107$ U/mg), IL-3 (specific activity $1{\cdot}4 \times 107$ U/mg) and GM-CSF (specific activity $1{\cdot}1 \times 10^5$ U/mg) all obtained from Glaxo IMB, Geneva, were used.

Statistical analysis

Statistical analysis was performed using the software Statview II and a Macintosh II CX. One factor analysis of variance (ANOVA) and regression analysis of the different values were used. The results are given in the legends to the figures and tables.

Results

Stimulation of human dermal fibroblast elastase activity by IL-1β

Pre-incubation of subconfluent fibroblast cultures with IL-1β at doses varying from 0·1 to 100 U/ml resulted in a dose-dependent increase in the elastase activity (Fig. 1). The optimal concentration of IL-1 of 10–100 U/ml induced a 70–100% enhancement of the activity of the enzyme. This stimulatory effect was detectable as early as 4 h after the addition of IL-1 to the culture and reached at plateau at 48 h (Fig. 2). The enhancement of elastase activity per mg of protein excluded the possibility that it resulted from an increase in protein synthesis induced by IL-1. Nor was it due to IL-1 alone as indicated by the absence of hydrolysis of Suc(Ala$_3$)NA without the fibroblasts. The increase in elastase activity appeared to be specific for IL-1 because IL-3 and GM-CSF were ineffective at the various doses tested.

Stimulation of fibroblast elastase activity by keratinocyte-conditioned medium

The conditioned medium obtained from the cultures of normal human keratinocytes (NHK) and from PAM 212 cells both enhanced the elastase activity of fibroblasts to a degree similar to that obtained using IL-1 (Table 1). A similar pattern of elastase induction was found with both

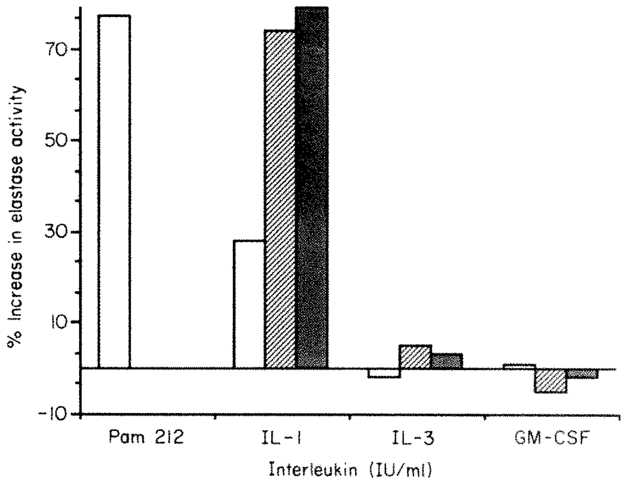

Figure 1. Effect of IL-1, IL-3, GM-CSF and of keratinocyte-derived soluble factors (from PAM 212 cells) on fibroblast elastase activity. Results (mean ± SEM of triplicates) of one out of three experiments are given; they are expressed as per cent increase in fibroblast elastase activity induced by the various samples compared to the elastase activity of unstimulated fibroblasts. The standard error of the mean was always below 5% and was omitted. □, 0·1 IU/ml; ▩, 10 IU/ml; ▨, 100 IU/ml.

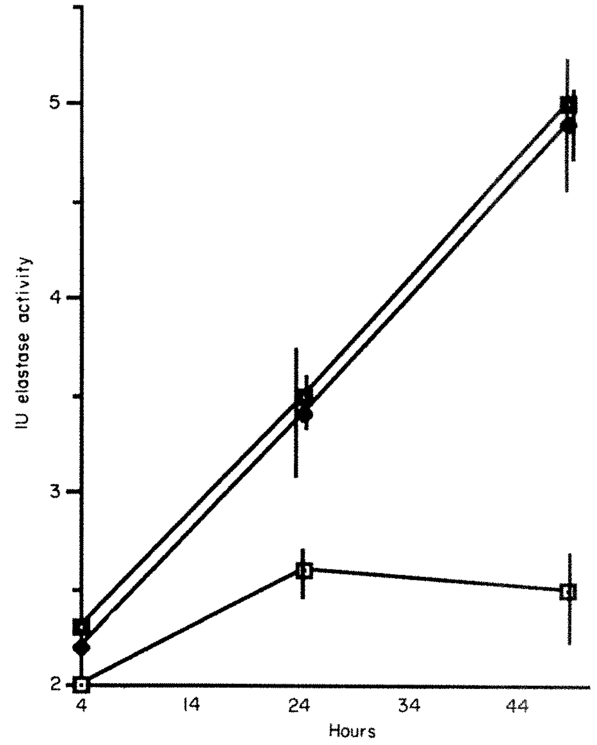

Figure 2. Kinetics of induction of elastase activity in response to control medium, IL-1β (100 IU/ml) or keratinocyte-derived supernatant. Results (mean ± SEM of duplicates) of one out of three experiments are given, expressed as international units (IU) of elastase activity. –⊡–, control; –◆–, Pam 212; ■, IL-1 (100 IU).

Table 1. Effect of epidermal cell culture supernatants and of rh IL-1 on fibroblast elastase activity. Results (mean±SEM of triplicates) are expressed as IU of elastase. Statistical analysis of the whole experiment using a one factor analysis of variance showed that the differences were statistically significant ($P=0{\cdot}0001$). Regression analysis of the values for IL-1-induced elastase activity showed that the differences were statistically significant ($P=0{\cdot}0001$)

				rh IL-1 beta (IU/ml)		
Cell type	Medium	NHK sup	PAM sup	0·1	10	100
F1	2·9±0·1	4·3±0·1 (+48%)	4·8±0·1 (+68%)	3 6±0·0 (+25%)	4 7±0·2 (+63%)	5·0±0 1 (+73%)
F2	8·1±0·4	11·5±0·6 (+43%)	10·9±1 (+35%)	ND	ND	ND
F3	2·7±0 1	4·7±0·1 (+74%)	4·8±0·2 (+76%)	3·4±0·1 (+26%)	5 3±0·3 (+96%)	5·1±0 1 (+88%)

NHK sup: supernatant from normal human keratinocyte culture; PAM sup: supernatant from PAM 212 cell culture

IL-1 and keratinocyte-conditioned medium (Fig. 2). The conditioned medium obtained from the cultures of normal human dermal fibroblasts without any IL-1 activity did not have any effect on elastase activity.

Discussion

Elastases are a group of metallo-proteases that are produced by several cell types including polymorphonuclear neutrophils (PMN), macrophages[13] and fibroblasts. This is a heterogeneous group because the substrate for PMN elastase is collagen type IV,[14] whereas the elastic fibres are the target for dermal fibroblast elastase.[15] Recent studies have shown that fibroblast elastase activity could be attributed to the joint action of an endopeptidase and an aminopeptidase for the *in-vivo* and *in-vitro* degradation of elastic fibres.[16] Little is known of the regulation of the synthesis of elastase by fibroblasts and several proteinase inhibitors produced by these cells could modulate their elastase activity.[17]

We have shown in this present study that recombinant IL-1β strongly stimulates the synthesis of elastase by normal human fibroblasts. Also, IL-1/ETAF produced by cultures of human keratinocytes has the same effect. This suggests that keratinocyte–fibroblast interactions *in vivo* could play an important role in affecting the metabolism of elastic fibres. Keratinocytes also produce an elastase inhibitor induced by spontaneous or experimental inflammation.[18] Thus epidermal cells secrete soluble factors that can regulate the metabolism of the elastic fibres, and can modulate the metabolism of collagen by the production of collagenase and collagenase inhibitor.[19] IL-1 could drive fibroblasts to produce proteases with the ultimate degradation of elastic fibres. This agrees with recent data that show that IL-1 plays an important role in the metabolism of the dermal extracellular matrix.[6] Because IL-1 is one of the major mediators of skin inflammation, our results also suggest that IL-1-induced stimulation of fibroblast elastases could be involved in the elastolysis observed in some post-inflammatory dermatoses as seen in acquired cutis laxa.

References

1 Dinarello CA. Biology of interleukin 1. *Faseb J* 1988; **2:** 108–15.

2 Schmidt JA, Mizel SB, Cohen D, Green I. Interleukin 1· a potential regulator of fibroblast proliferation. *J Immunol* 1982; **128:** 2177–82.

3 Dayer JM, De Rochemonteix B, Burrus B *et al.* Human recombinant interleukin 1 stimulates collagenase and prostaglandin E2 production by human synovial cells. *J Clin Invest* 1986; **77:** 645–8.

4 Postlethwaite AE, Lachman LB, Mainardi CL, Kang AH. Interleukin 1 stimulation of collagenase production by cultured fibroblasts. *J Exp Med* 1983; **157:** 801–6.

5 Duncan MR, Berman B. Differential regulation of collagen, glycosaminoglycan, fibronectin and collagenase activity production in cultured human adult dermal fibroblasts by interleukin 1-alpha and beta and tumor necrosis factor alpha and beta. *J Invest Dermatol* 1989; **92:** 699–706.

6 Luger TA, Stalder BM, Katz SI, Oppenheim JJ. Epidermal cell (keratinocyte) derived thymocyte activating factor. *J Immunol* 1981; **127:** 1493–8.

7 Sauder DN, Carter CS, Katz SI, Oppenheim J Epidermal cell production of thymocyte activating factor (ETAF). *J Invest Dermatol* 1982; **79:** 34–9.

8 Hauser C, Saurat JH, Schmitt A *et al.* Interleukin 1 is present in normal human epidermis. *J Immunol* 1986; **136:** 3317–23.

9 Delaporte E, Vincent C, Bonnefoy JY *et al.* Keratinocyte–fibroblast interactions: cultured human keratinocytes produce soluble factors stimulating fibroblast proliferation. Relationship to IL-1. *Path Biol* 1989; **37:** 875–80.

10 Green H, Kehinde O, Thomas J. Growth of cultural human epidermal cells into multiple epithelia suitable for grafting. *Proc Natl Acad Sci (USA)* 1979; **76:** 5665–8.

11 Bieth J, Spiess B, Wermuth CG. The synthetic and analytical use of a highly sensitive and convenient substrate of elastase. *Biochem Med* 1974; **11:** 350–7.

12 Szendroi M, Meimon G, Bakala H *et al.* On the presence of a metalloprotease in human skin fibroblasts that degrades the

human skin elastic fiber system. *J Invest Dermatol* 1984; **83:** 224–9.

13 Lammers AM, Van De Kerkhof PCM, Schalkwijk J, Mier PD. Elastase, a marker for neutrophils in skin infiltrates. *Br J Dermatol* 1986; **115:** 181–6.

14 Briggaman RA, Schechter NM, Fraki J, Lazarus GS. Degradation of the epidermal–dermal junction by proteolytic enzymes from human skin and human polymorphonuclear leukocytes. *J Exp Med* 1984; **160:** 1027–42.

15 Schwartz DE, Paller AS, Lizak PP, Pearson RW. Elastase and neutral cathepsin production by human fibroblasts: Effect of culture conditions on synthesis and secretion. *J Invest Dermatol* 1986; **86:** 63–8.

16 Homsy R, Pelletier-Lebon P, Tixier JM *et al.* Characterization of human skin fibroblasts elastase activity. *J Invest Dermatol* 1988; **91:** 472–7.

17 Reynolds JJ, Brunning RA, Cawston TE, Murphy G. Tissue metalloproteinase inhibitors and their role in matrix catabolism. In: *Cellular Interactions.* (Dingle J., Gordon J., eds). North-Holland/Elsevier: Amsterdam: 1981; 205–213.

18 Schalkwijk J, Lammers AM, Chang A *et al.* An epidermal elastase inhibitor induced by spontaneous or experimental inflammation. *J Invest Dermatol* 1988; **91:** 376A.

19 Petersen MJ, Woodley DT, Stricklin GP, O'Keefe EJ. Constitutive production of procollagenase and tissue inhibitor of metalloproteinases by human keratinocytes in culture. *J Invest Dermatol* 1989; **92:** 156–9.

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Protein kinase C activity in normal and psoriatic cells: cultures of fibroblasts and lymphocytes

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Summary

Protein kinase C (PKC) activity was measured in cultures of fibroblasts from biopsies of the involved and uninvolved skin of seven patients with psoriasis and from the skin biopsies of nine normal controls. PKC activity was significantly increased ($P<0{\cdot}005$) in the particulate fraction of fibroblasts obtained from the involved areas of skin (450 ± SEM 89 pmol/mg protein/3 min) and the uninvolved skin (394 ± 94 pmol/mg protein/3 min) in psoriasis as compared to that of controls (103 ± 24 pmol/mg protein/3 min). The soluble fraction of PKC activity was comparable in controls and in the fibroblasts obtained from involved areas and not significantly different from the values in fibroblasts from uninvolved skin. PKC activity was also measured in the soluble and particulate fractions of lymphocytes from 13 patients with psoriasis and from 14 normal controls. The PKC activity did not differ in the lymphocytes of patients with psoriasis from the controls in either the cytosolic or the membrane fractions.

The increase in PKC activity as expressed at the membrane level of psoriatic fibroblasts may be related to an increase in sensitivity of these cells to hormones or growth factors involved in the regulation of their growth.

The lesions of psoriasis represent an example of benign hyperproliferation with abnormal differentiation.[1,2] Recent studies suggest that the dermis plays an important role in the development of the lesions in psoriasis.[3] It has been suggested that there is an abnormal growth of fibroblasts in the disorder[4–6] and it is of interest to study the various factors that can modulate their growth and differentiation. These factors include cyclic AMP and calcium (Ca^{2+}) which have been implicated in the regulation of cell growth and in the differentiation of cells as well as in malignant transformation.[7,8] The cAMP and Ca^{2+} appear to act through different protein kinases with cAMP mediated by protein kinase A (PKA).[9,10] We have shown that PKA is decreased in psoriatic cells both in cultured fibroblasts and in circulating red blood cells. The levels of PKA are also correlated with the severity of the disease.[11,12] One of the mediators is the Ca^{2+} and phospholipid-dependent protein kinase C (PKC). This is present in various tissues and mediates the action of hormones, growth factors and oncogene products.[13–16] Recent studies have described altered levels of PKC activity in psoriatic epidermis and the fibroblasts in patients with psoriasis, suggesting a role for this enzyme in the pathology.[17,18]

We have studied the activity of PKC in cultures of fibroblasts from the involved and uninvolved areas of skin of patients with psoriasis and from the skin of normal controls. Activity has also been measured in circulating blood cells from patients with psoriasis and from normal controls.

Methods

Patients

Fourteen healthy controls (five males, nine females) aged 22–45 years (mean 35 ± 2 years) and 13 patients with psoriasis (six males, seven females) aged 18–50 years (mean 38 ± 3 years) were studied. The protein kinase C activity of circulating lymphocytes of patients and controls was measured, as was that of cultured fibroblasts from seven of the patients and from nine controls. All the biopsies were taken from the skin on the buttock.

Materials

Trypsin, collagenase (type II 5), histone type V, leupeptin, ATP, phosphatidyl serine and diolein were all obtained from Sigma. (^{32}P)ATP was from Amersham and Dulbecco medium and glutamine were from Flow

Laboratories. Fetal calf serum was from Seromed (Munich, Germany).

Cell cultures

Cell cultures of human fibroblasts were obtained from skin biopsies taken from patients with psoriasis from both uninvolved and involved tissue. The fibroblasts were cultured in Dulbecco medium containing 10% fetal calf serum, 50 U/ml penicillin and 50 μg/ml of streptomycin and were plated on to a 100-mm cell culture dish. The medium was changed every 3 days. For protein kinase determinations the cells were used at their fourth or fifth passages.

Lymphocyte preparations

The blood samples were collected in tubes containing liquemin and were diluted in the same volume of Hank's solution (Gibco). Lymphocytes were separated in Ficoll Plaque (Pharmacia) by centrifugation at 600 *g* for 30 min. The clear ring of lymphocytes was collected with a Pasteur pipette and centrifuged at 400 *g* for 50 min. The pellet of lymphocytes was then resuspended and washed three times with Hank's solution.

Preparation of cellular fraction

All procedures were performed at 0–4°C. The cells were washed three times with phosphate-buffered saline and then harvested into homogenization buffer (20 mM Tris HCl pH 7·5, 2 mM EDTA, 0·33 M sucrose and 20 μg/ml leupeptin) and were then disrupted in a Dounce homogenizer. The homogenate was centrifuged at 800 *g*, for 10 min and the supernatant then centrifuged at 12,000 r.p.m. for 10 min in a microfuge. The supernatant was then used as the soluble fraction. The pellet (crude membrane fraction) was rehomogenized with solubilization buffer (20 mM Tris pH 7·5 , 2 mM EDTA, NP40 1% and 20 μg/ml leupeptin). After 1 h incubation at 4°C the solution was centrifuged for 5 min at 12,000 r.p.m. The supernatant (solubilized particulate fraction) was used as the source for the membrane-bound activity of PKC.

Protein kinase assay

The supernatant or the solubilized particulate fraction was loaded on DEAE cellulose columns (DE 52, Whatman 0·7 × 3 cm) equilibrated with Tris buffer and the columns were then washed with 10 ml of the buffer followed by 1·8 ml 0·05 M NaCl in the same buffer. PKC activity was then eluted with 2 ml 0·1 M NaCl and the activity determined by measuring the transfer of ^{32}P from (γ^{32}P)ATP to histone. The reaction was initiated by the addition of 50 μl of enzyme preparation to a reaction mixture containing 20 mM Tris HCl pH 7·5, 0·75 mM $CaCl_2$, 10 mM Mg acetate, 100 mM (γ^{32}P)ATP (100 c.p.m./pmol), 25 μg histone and 16 μg/ml leupeptin, with or without 24 μg phosphatidyl serine and 0·8 μg diolein. Phosphatidyl serine and diolein were evaporated under nitrogen gas and sonicated in 5 ml Tris HCl pH 7·5 for 3 min. Phospholipids were added to the reaction mixture just prior to the initiation of the reaction by the action of the enzyme. After incubation at 30°C for 3 min the reaction was terminated by the addition of 1 ml 25% trichloroacetic acid and the precipitate was collected by filtration over a millipore filter (0·45 μm). Filters were washed eight times/3 ml/wash with 5% trichloroacetic acid, dissolved in HP/b scintillation fluid and counted for radioactivity. Triplicate determinations agreed within ± 10%. To determine specific PKC activity the amount of ^{32}P incorporation in the absence of added phospholipids was subtracted from the amount of ^{32}P incorporation into histone in the presence of phospholipids.

Protein determination

Protein concentration was determined by fluorimetric assay using bovine serum albumin as the standard.[19]

PASI score

The severity of the psoriasis was assessed as a PASI score as previously described.[11,20]

Results

The PKC activity was measured and compared in both the soluble and particulate fractions of normal and psoriatic fibroblasts that were subconfluent ($2{\cdot}5 \times 10^6$ cells/10-cm dish). This was of importance because PKC activity in the soluble and particulate fractions alters with cell density (Fig. 1). With increasing cell density there is an increase of PKC activity in the cytosol and a progressive decrease is noted on the plasma membranes. Growth-arrested cells at high density possess low membrane-associated PKC activity but in the soluble fraction this was markedly elevated over that noted in a rapidly growing population of cells.

The values of PKC activity in the particulate fractions of fibroblasts grown to subconfluency from biopsies taken from the involved and uninvolved skin of eight

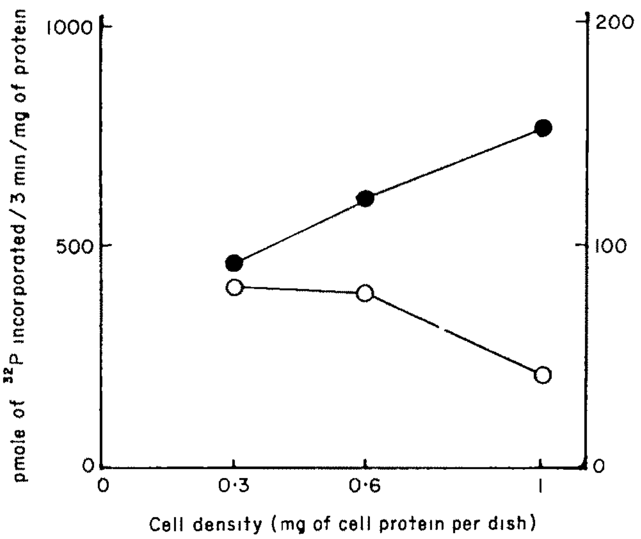

Figure 1. Variation of PKC activity in cytosol and membrane of normal fibroblasts with cell density. C kinase activity is expressed in pmole of ^{32}P incorporated per 3 min/mg protein, and the cell density in mg cell protein per dish. ●, cytosol; ○, membranes.

patients with psoriasis and from nine control subjects are shown in Table 1. PKC activity was increased in the membrane fraction of psoriatic fibroblasts and the mean was 450±SEM 89 pmol/mg protein/3 min. It was significantly increased in the membrane fraction of fibroblasts from uninvolved skin in psoriasis, 394±SEM 94 pmol/mg protein/3 min as compared to the controls, 103·6±SEM 24 pmol/mg protein/3 min. There was no significant difference in the PKC activity of fibroblasts from involved and uninvolved skin in the psoriatic patients in the particulate fraction.

In the soluble fraction, PKC activity in the fibroblasts from the involved skin of the patients with psoriasis, 405±SEM 70 pmol/mg protein/3 min was similar to that of the controls, 393±SEM 66 pmol/mg protein/3 min. PKC activity measured in the cytosol of the fibroblasts derived from uninvolved tissue from four patients with psoriasis was lower, 268±SEM 71 pmol/mg protein/3 min, but was not statistically different from the controls.

The levels of PKC activity in lymphocytes from patients with psoriasis as compared with normal controls are shown in Table 2. The difference found was not statistically significant.

Discussion

We have studied PKC activity in the particulate fractions at low cell density, and the increase of PKC activity in the cytosol of a growth-arrested population of cells. The subcellular distribution of PKC between soluble and particulate fractions is of importance. On stimulation of the cells, for example by hormones, growth factors or phorbol esters, the cytoplasmic PKC becomes associated with the plasma membranes where it is activated.[21–23] This alteration in the subcellular distribution of PKC may be an important regulatory mechanism to allow preferential calcium-mediated phosphorylation of specific endogenous protein substrates, thus modulating calcium effects on such cellular processes as growth rate and morphology.

In agreement with a previous study,[18] we found an increased PKC activity in the membranes of psoriatic fibroblasts as compared to controls. PKC activity in the

Table 1. Values of PKC activity in soluble and particulate fractions of normal (nine subjects) and psoriatic (eight patients) fibroblasts. The severity of psoriatic disease is expressed by the PASI score (cutaneous index). UIS, uninvolved skin; IS, involved skin. The results are expressed in pmoles of ^{32}P incorporated/mg protein/3 min

Normal			Psoriatic			
Subjects	Soluble	Particulate	Patients	Soluble IS/UIS	Particulate IS/UIS	Cutaneous score
1	83	62	A	522/442	372/636	23
2	346	140	B	240/181	574/83	6·5
3	470	256	C	504/322	743/507	6·4
4	422	58	D	626/—	128/—	16
5	480	—	E	529/—	321/—	20·2
6	782	70	F	288/126	685/303	15
7	196	97	G	126/—	111/—	65
8	310	102	H	—/—	671/442	9·5
9	450	46				
Mean±SEM	393±66	103·6±24		405±70/268±71	450·6±89/394·2±94	

Table 2. Values of PKC activity in soluble and particulate fractions of normal (14 subjects) and psoriatic (13 patients) lymphocytes. The results are expressed in pmol of ^{32}P incorporated/mg protein/3 min

	Normal			Psoriatic		
Subjects	Soluble	Particulate	Patients	Soluble	Particulate	Cutaneous score
1	4007	3772	A	5688	4973	23
2	2116	3504	B	796	691	6·5
3	5391	4297	C	5158	3274	6·4
4	3933	3088	D	1810	3947	16
5	806	2046	E	1746	1754	20·2
6	912	1959	F	1091	2824	15
7	337	809	G	—	—	65
8	1563	1356	H	2320	1842	9·5
9	542	1039	I	554	818	9·5
10	1876	4440	J	234	3383	23·4
11	2520	3320	K	4427	4127	19·3
12	1039	2320	L	3848	2998	9·6
13	873	1696	M	1290	819	17·6
14	1353	2218	N	1251	1837	3
Mean ± SEM	1948 ± 390	2561 ± 305		2349 ± 417	2368 ± 326	

cytosol of fibroblasts of psoriatic patients was not significantly different when compared to that in the normal controls. These results are not in agreement with another study[17] where a decreased PKC activity in fragments of epidermis from psoriatic subjects was found. The decreased PKC activity might be related to a down-regulation mechanism of PKC or a decrease in the synthesis of the enzyme. The PKC activity in the fibroblasts seemed to vary with the severity of the disease, and this might suggest that PKC activity in the membranes of psoriatic fibroblasts is first activated, before being down-regulated with the more severe disease. However, the values observed in patients with a high cutaneous score were also comparable to that found in normal subjects. On the other hand, increased activity of PKC at the membrane level suggests a greater sensitivity to hormones and growth factors. We recently demonstrated an increased sensitivity of psoriatic fibroblasts to platelet-derived growth factor.[24] The highest PKC activity was observed in the membrane of rapidly growing fibroblasts and was decreased in growth-arrested cell (Fig. 1).

The alterations in PKC activity in the fibroblasts was not found in the lymphocytes. There was no correlation of the PKC activity with the severity of the psoriasis, in contrast to what was found with fibroblasts.[25]

Our studies have demonstrated alterations in the levels of both PKC and PKA activities in fibroblasts from patients with psoriasis. These changes may result in an abnormal phosphorylation of specific mediators in the regulation of growth of the fibroblasts in psoriasis.[11,12]

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References

1 Voorhees JJ. Commentary: cyclic adenosine monophosphate regulation of normal and psoriatic epidermis. *Arch Dermatol* 1982; 118: 869–74.

2. Krueger GG, Bergstresser PR, Lowe NJ *et al.* Psoriasis. *J Am Acad Dermatol* 1984; 11: 937–47.

3 Saiag P, Coulomb B, Lebreton C *et al.* Psoriatic fibroblasts induce hyperproliferation of normal keratinocytes in a skin equivalent model *in vitro*. *Science* 1985; 230: 669–72.

4 Priestley GC, Adams LW. Hyperactivity of fibroblasts cultured from psoriatic skin. I. Faster proliferation and effect of serum withdrawal. *Br J Dermatol* 1983; 109: 149–56.

5 Baden HP, Kubilus J, MacDonald MJ. Normal and psoriatic keratinocytes and fibroblasts compared in culture. *J Invest Dermatol* 1981; 76: 53–5.

6 Priestley GC, Adams LW, Oakley CA, Prescott RJ. Proliferative responses of fibroblasts from psoriatic and normal skin to clobetasol propionate. *Acta Derm Venereol (Stockh)* 1983; 63: 393–6

7 Berridge MJ. The interaction of cyclic nucleotides and calcium in the control of cellular activity. *Adv Cycl Nucleotide Res* 1975; 6: 1–98.

8 Rasmussen H, Goodman DBP. Relationships between calcium and cyclic nucleotides in cell activation. *Physiol Rev* 1977; 57: 421–509.

9 Kuo JF, Greengard P. Cyclic neucleotide dependent protein kinases. IV. Widespread occurrence in adenosine 3′5′monophosphate-

dependent protein kinase in various tissues and phyla of the animal kingdom. *Proc Natl Acad Sci USA* 1969; **64:** 1349–55.

10 Walsh DA, Ashby CD. Protein kinases: aspects of their regulation and diversity. *Rec Prog Horm Res* 1973; **29:** 329–59.

11 Evain Brion D, Raynaud F, Plet A *et al.* Deficient cyclic AMP dependent protein kinases in human psoriasis. *Proc Natl Acad Sci USA* 1986; **83:** 5272–6.

12 Raynaud F, Leduc C, Anderson WB, Evain Brion D Retinoid treatment of human psoriatic fibroblasts induces an increase in cyclic AMP dependent protein kinase activity. *J Invest Dermatol* 1987; **89:** 105–10.

13 Takai Y, Kishimoto A, Iwasa Y *et al.* Calcium dependent activation of a multifunctional protein kinase by membrane phospholipids. *J Biol Chem* 1979; **254:** 3692–5.

14 Pearon CW, Tashjian AH Jr. Thyrotropin releasing hormone induces redistribution of protein kinase C in GH_4C_1 rat pituitary cells. *J Biol Chem* 1985; **260:** 8366–371.

15 Cochet C, Gill GN, Meisenhelder J *et al.* C kinase phosphorylates the epidermal growth factor receptor and reduces its epidermal growth factor stimulated tyrosine protein kinase activity. *Biol Chem* 1984; **259:** 2553–8.

16 Sugitomo Y, Whitman M, Cantley LC, Erickson RL. Evidence that the Rous sarcoma virus transforming gene product phosphorylates phosphatidylinositol and diacylglycerol. *Proc Natl Acad Sci USA* 1984; **81:** 2117–21.

17 Horn F, Marks F, Fisher G *et al.* Decreased protein kinase C activity in psoriatic versus normal epidermis. *J Invest Dermatol* 1987; **88:** 220–2.

18 Nagao S, Seishima M, Mori S, Nozawa Y. Increased protein kinase C activity in fibroblast membranes from psoriatic patients. *J Invest Dermatol* 1988; **90:** 406–8.

19 Bohlen P, Stain S, Dairman W, Unfriend S. Fluorometric assay of proteins in the nanogram range. *Arch Biochem Biophys* 1973; **155:** 213–20.

20 Frederiksson AJ, Peterssonn BC. Severe psoriasis-oral therapy with new retinoid. *Dermatologica* 1978; **157:** 238–44.

21 Kraft AS, Anderson WB. Phorbol esters increase the amount of Ca^{2+} phospholipid dependent protein kinase associated with plasma membranes. *Nature* 1983, **301:** 621–3.

22 Vilgrain I, Cochet C, Chambaz EM. Hormonal regulation of a calcium activated phospholipid dependent protein kinase in bovine adrenal cortex. *J Biol Chem* 1984; **259:** 3403–6.

23 Farrar WL, Anderson WB. Interleukin 2 stimulation of protein kinase C with plasma membrane. *Nature* 1985; **315:** 233–5.

24 Gu XF, Raynaud F, Evain Brion D. Increased chemotactic and mitogenic response of psoriatic fibroblasts to platelet-derived growth factor. *J Invest Dermatol* 1988; **91:** 599–602.

25 Raynaud F, Liapi C, Gerbaud P *et al.* Effect of retinoic acid on cAMP dependent protein phosphorylation in psoriatic fibroblasts. *Biochem Biophys Res Commun* 1988; **156:** 263–8.

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Neuropeptides in skin disease: increased VIP in eczema and psoriasis but not axillary hyperhidrosis

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Summary The neuropeptides vasoactive intestinal polypeptide (VIP), substance P and somatostatin were studied in skin biopsies from patients with eczema, psoriasis and axillary hyperhidrosis. VIP concentrations were elevated in skin affected by eczema and psoriasis, whereas substance P and somatostatin levels did not differ from controls. There was a higher concentration of VIP, but not of substance P or somatostatin, in normal axillary skin when compared to adjacent trunk skin, with abundant VIP-containing fibres surrounding eccrine sweat glands. The VIP concentration was unchanged in skin affected by axillary hyperhidrosis. VIP may increase local blood flow in eczema and psoriasis, but does not appear to play a role in axillary hyperhidrosis.

A number of neuropeptides have been demonstrated in cutaneous nerves:[1–7] substance P and somatostatin are present in sensory fibres, while vasoactive intestinal polypeptide (VIP) coexists with acetylcholine in post-ganglionic sympathetic fibres. Substance P is a vasodilator in skin, and may mediate the axon reflex flare response, as may other neuropeptides such as calcitonin gene-related peptide (CGRP), VIP and somatostatin.[8,9] VIP is a potent vasodilator, and may augment sweat secretion.[1,10] Peripheral tissue injury and inflammation results in altered expression of neuropeptides, which may play a role in pathological processes.[11,12] Neuropeptides may also serve as selective markers of nerve fibre loss and regeneration, and act as trophic agents. We have studied the neuropeptides VIP, substance P and somatostatin in affected skin from patients with eczema, psoriasis and axillary hyperhidrosis.

Methods

Full thickness biopsies (0·3–0·7 g) of affected non-digital forearm skin were taken, with informed consent, from patients with atopic eczema ($n=15$, age range 24–45 years), chronic psoriasis ($n=9$, age range 30–55 years) and axillary hyperhidrosis ($n=6$, age range 28–42 years), using a local anaesthetic. The patients had not taken any medication for at least a week prior to biopsy. Seven biopsies of normal non-digital forearm and periaxillary skin ($n=7$) were obtained in the same manner with informed consent from age-matched subjects undergoing surgical operations for unrelated disorders. For radioimmunoassay, the biopsies were frozen within minutes of collection and stored at −70°C. The peptide content was extracted by boiling the tissues in 0·5 M acetic acid (1:10, w/v), and the extracts assayed for substance P, somatostatin and VIP using previously described specific assays.[2] The sensitivity of each assay was 0·3 fmol per assay tube. Chromatographic analysis of pooled samples from normal skin showed that the VIP immunoreactivity co-eluted with pure porcine VIP, the substance P immunoreactivity eluted as a single peak corresponding to synthetic substance P, and the somatostatin immunoreactivity eluted as two peaks corresponding to somatostatin-14 and somatostatin-28. For immunocytochemical studies, fresh tissue was fixed in a 0·4% solution of *p*-benzoquinone in phosphate-buffered saline, and cryostat sections (7 μm thick) stained by a modification of the indirect immunofluorescent method, as previously described.[7] Tissue was not available from the patients with eczema and psoriasis for immunocytochemical studies. Neuropeptide concentrations were compared using Student's unpaired *t*-test.

Results

The concentrations of neuropeptides in all tissue extracts are shown in Table 1. A small but significant increase of

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Table 1. Neuropeptide concentrations in skin biopsies (pmol/g, mean ± SEM)

	VIP	Substance P	Somatostatin
Normal	0·6±0·2	1·3±0·2	1·0±0·3
Eczema	1·4±0·3*	1·6±0·3	1·1±0·4
Psoriasis	1·5±0·3*	1·3±0·4	1·0±0·3
Normal axilla	5·5±0·7**	1·2±0·2	0·9±0·3
Normal periaxilla	0·7±0·3	1·4±0·4	1·1±0·3
Axillary hyperhidrosis	4·5±0·8	1·5±0·5	0·8±0·3

*$P<0{\cdot}05$, Student's *t*-test, compared to normal skin: **$P<0{\cdot}001$, Student's *t*-test, compared to normal periaxillary skin.

VIP content was seen in biopsies of skin affected by eczema and psoriasis ($P<0{\cdot}05$); substance P and somatostatin content showed no change.

The concentrations of substance P and somatostatin were similar in trunk and axillary skin, whereas the VIP concentration was significantly higher in axillary skin when compared to adjacent trunk skin ($P<0{\cdot}001$). VIP immunoreactive nerve fibres were seen in abundance around eccrine sweat glands in axillary skin (Fig. 1a), with a few fibres adjacent to apocrine glands (Fig. 1b), sweat ducts and blood vessels. Nerve fibres containing substance P and somatostatin were seen as beaded fibres running through the dermis, some apparently ending freely or in close relation with arterioles, whereas a few fibres extended to the epidermis, reaching almost up to the stratum corneum. There appeared to be no change in the neuropeptide innervation in axillary skin from patients with axillary hyperhidrosis as assessed by both radioimmunoassay and immunocytochemistry.

Discussion

VIP concentrations are increased in eczema and psoriasis, but the significance of this is unknown. Further studies, using immunocytochemistry, are necessary to localize the increase of VIP. Substance P, somatostatin and VIP have all been considered as candidates for mediators of the triple response in human skin,[8,9] and injured sensory and sympathetic fibres increase their expression of VIP.[13] The elevated VIP levels may increase local blood flow in these conditions.

Previous studies have suggested that increased numbers of substance P-immunoreactive fibres may be seen in psoriatic skin lesions,[14] and substance P-like immunoreactivity may be increased in blister fluid from inflamed skin.[15] These reports discussed the mechanisms whereby substance P may affect these conditions. Although our study fails to support a role for substance P in psoriasis and eczema, further studies of substance P turnover are necessary to exclude such a role.

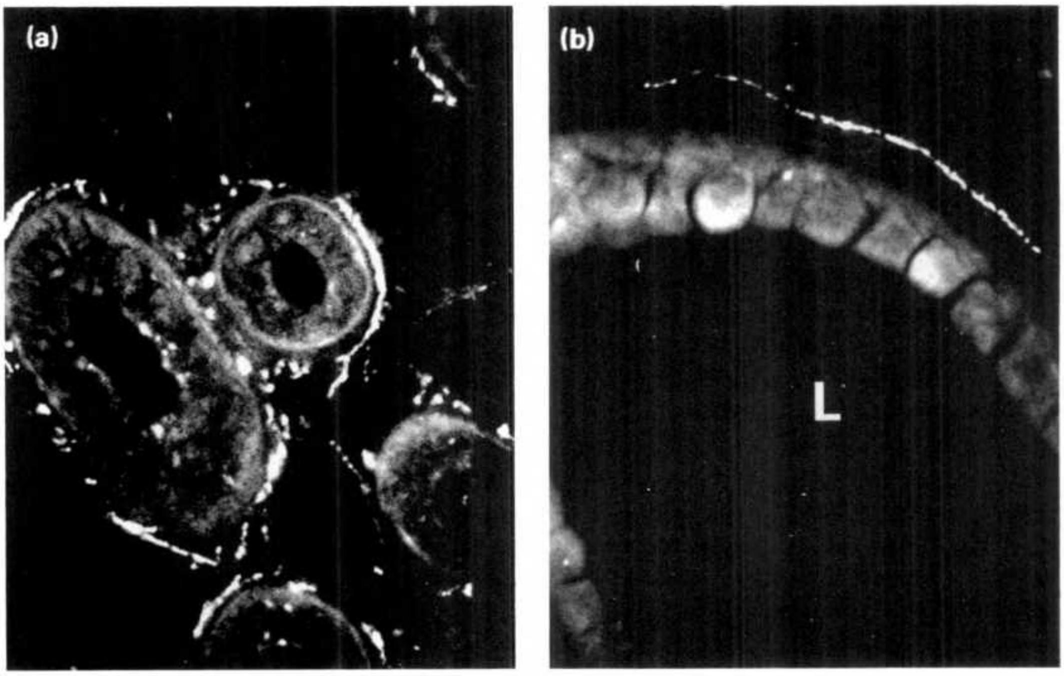

Figure 1. Immunoreactivity for VIP in axillary skin. Abundant VIP-immunoreactive nerve fibres are seen around (a) eccrine sweat glands, but are present only sparsely around (b) apocrine glands (L, lumen). (Magnification: (a) ×410, (b) ×450.)

The high concentration of VIP in axillary skin is the result of its presence in post-ganglionic sympathetic fibres to eccrine sweat glands. It is of interest that, whereas VIP concentration is below the assay detection limit in cat trunk skin (<0·05 pmol/g), it is very high in the footpads (29 pmol/g),[2] which is the only place where cats have eccrine sweat glands.[16] Stimulation of the lumbar sympathetic ganglia of the cat is known to cause sweat secretion and the release of acetylcholine and VIP from the footpad skin.[1] Local infusion of VIP alone causes vasodilation but no sweat secretion, although it does enhance sweat secretion induced by acetylcholine infusion.[10] A recent study has quantified VIP in human axillary skin and sweat, and demonstrated VIP binding sites on eccrine gland secretory cells.[17]

Axillary hyperhidrosis has been attributed to overactivity of eccrine, not apocrine glands.[18] These glands are histologically normal, as is the acetylcholinesterase activity in the nerve fibres innervating them. This study, of skin taken from patients with severe axillary hyperhidrosis, suggests that the VIP content is not responsible for the condition. This result does not exclude a change in local VIP turnover: however, in general, neuropeptide levels reflect their expression and activity.[13]

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References

1 Lundberg JM. Evidence for coexistence of VIP and acetylcholine in neurons of cat exocrine glands. Morphological, anatomical and functional studies. *Acta Physiol Scand Suppl* 1981; **496**: 1–57.

2 O'Shaugnessy DJ, McGregor GP, Ghatei MA *et al.* Distribution of bombesin, somatostatin, substance P and vasoactive intestinal polypeptide in feline and porcine skin. *Life Sci* 1983; **32**: 2827–36.

3 Dalsgaard CJ, Jonsson CE, Hokfelt T, Cuello AC. Localisation of substance P immunoreactive nerve fibres in human digital skin. *Experientia* 1983; **39**: 1018–20.

4 Bloom SR, Polak JM. Regulatory peptides in the skin. *Clin Exp Dermatol* 1983; **8**: 3–18.

5 Johansson O. A detailed account of NPY immunoreactive nerves and cells of human skin. Comparison with VIP, substance P and PHI containing structures. *Acta Physiol Scand* 1986; **128**: 147–53.

6 Tainio H, Vaalasti A, Rechardt R. The distribution of sympathetic, adrenergic, tyrosine hydroxylase- and neuropeptide Y-immunoreactive nerves in human axillary seat glands. *Histochemistry* 1986, **85**: 117–20.

7 Karanth SS, Springall DR, Lucas S *et al.* Changes in nerves and neuropeptides in skin from 100 leprosy patients investigated by immunocytochemistry. *J Pathol* 1989; **157**: 15–26.

8 Hägermark Ö, Hokfelt T, Pernow B. Flare and itch induced by substance P in human skin. *J Invest Dermatol* 1978; **71**: 233–5.

9 Anand P, Bloom SR, McGregor GP. Topical capsaicin pretreatment inhibits axon reflex vasodilation caused by somatostatin and VIP in human skin. *Br J Pharmac* 1983; **78**: 665–9.

10 Fahrenkrug J. VIP as a neurotransmitter in the peripheral nervous system. In: *Vasoactive Intestinal Polypeptide* (Said S, ed). New York: Raven Press, 1982; 361–72.

11 Senapati A, Anand P, McGregor GP *et al.* Depletion of neuropeptides during wound healing in rat skin. *Neurosci Lett* 1986; **71**: 101–5.

12 Helme RD, Andrews PV, Watson BA. Neurogenic inflammation caused by wool fabric in rat: possible mediation by substance P. *Neurosci Lett* 1986; **71**: 333–7.

13 Anand P, Gibson SJ, Scaravilli F *et al.* Studies of VIP expression in injured peripheral neurons using capsaicin, sympathectomy and mf mutant rats. *Neurosci Lett* 1990 (in press).

14 Naukkarinen A, Nickoloff BJ, Farber EM. Quantification of cutaneous sensory nerves and their substance P content in psoriasis. *J Invest Dermatol* 1989; **92**: 126–9.

15 Wallengren J, Ekman R, Möller H. Substance P and vasoactive intestinal polypeptide in bullous and inflammatory skin disease. *Acta Derm Venereol (Stockh)* 1986; **66**: 23–8

16 Montagna W. Comparative anatomy and physiology of the skin. *Arch Dermatol* 1967; **96**: 357–63.

17 Eedy DJ, Shaw C, Armstrong EP *et al.* Vasoactive intestinal polypeptide and peptide histidine methionine in human eccrine sweat glands: demonstration of innervation, specific binding sites and presence in secretions. *Br J Dermatol* 1990; **123**: 65–76.

18 Hurley HJ, Shelley WB. Axillary hyperhidrosis. Clinical features and local surgical management. *Br J Dermatol* 1966, **78**: 127–40.

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Determination of released CD4 and CD8 antigens in the suction-blister fluid and horny-tissue extract in patients with psoriasis

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Summary The amounts of soluble CD4 and CD8 antigens were measured in suction-blister fluid and extracts of horny tissue in patients with psoriasis. The levels of soluble CD4 and CD8 in the suction-blister fluid of lesional skin in psoriasis was significantly higher than from normal skin and the uninvolved skin in psoriasis. Levels of soluble CD4 and CD8 in the extracts of horny tissue in psoriasis were significantly higher than those from non-psoriatic skin.

Psoriasis is an inflammatory skin disorder with alterations in T lymphocyte-related functions.[1] Immunohistochemical studies have shown that in the early lesions of psoriasis there is an infiltration of activated helper T cells in the epidermis and that late lesions are characterized by an influx of suppressor T lymphocytes.[2,3] Involvement of helper T cells has been suspected by the induction of psoriasis by interleukin 2[4] and the beneficial effect of cyclosporin.[5]

The presence of CD4 antigen defines a subset of mature T lymphocytes that possess both regulatory (helper) and antigen-recognition functions that are restricted by class II HLA antigens.[6,7] This contrasts with the other T-cell subsets that express CD8 antigen, which has cytotoxic functions and are HLA class I restricted.[8,9] These surface antigens are released extracellularly and permit the monitoring of $CD4^+$ and $CD8^+$ cell activity in various pathological events.[10,11] A soluble form of CD4 has been shown to inhibit HIV-1 virus infection.[12,13] A soluble form of CD8 is also present in the supernatant of $CD8^+$ T-cell leukaemia cell lines.[14] These secreted molecules may have immunoregulatory roles, although very little has so far been reported about their action in psoriasis.[15] In the present study we have measured the levels of soluble CD4 and CD8 in the suction-blister fluid and from extracts of horny tissue obtained from lesional and non-lesional skin in psoriasis.

Methods

Suction-blister formation

Fifteen patients with psoriasis (11 males, four females; age range 18–59 years, mean 34 years) and 14 healthy male volunteers (age range 19–23 years, mean 21 years) were studied. None of the patients with psoriasis had any treatment for at least 2 weeks before the study. Suction blistering was carried out on the normal uninvolved skin as well as on plaques of psoriasis with a diameter of 1–2 cm and lesions of pustular psoriasis larger than 3 cm in diameter. The blisters were produced using attachment devices with hollow syringes whose broad and flat ends were placed on the skin and a negative pressure applied for 2–3 h of 200–300 mmHg. The blister fluid was collected and following centrifugation at 1600 *g* for 15 min the supernatant was then stored at $-70°C$. Only those blister fluids from psoriatic involved skin with little blood contamination were used for this study.[16]

Extracts of horny tissue

Scale was collected from skin lesions from nine patients with psoriasis vulgaris, two patients with erythrodermic psoriasis and nine patients with pustular psoriasis and four patients with palmoplantar psoriasis (PPP). The controls used were hyperkeratotic tissue from the plantar callus from eight patients and scale from four patients with ichthyosis vulgaris and one patient with pityriasis rubra pilaris. The water-soluble components from the horny tissue were extracted in phosphate-buffered saline at pH 7·4 and containing 100 μg/ml streptomycin.[16]

Molecular sieve chromatography

TSK-G2000SW exclusion high-performance liquid column chromatography (Tosoh, Tokyo, Japan; $7{\cdot}5 \times 30$ cm) was equilibrated with phosphate-buffered saline (PBS) and calibrated with dextran blue (2000 kDa), γ-

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globulin (158 kDa), bovine serum albumin (67 kDa), ovalbumin (44 kDa) and carbonic anhydrase (29 kDa). After injecting 0·5 ml of suction-blister fluid or horny-tissue extract into the column, the column was eluted with PBS and fractions of 0·5 ml were collected. The protein content was estimated by the measurement of absorbance at 280 nm. The fractions were assayed for soluble CD4 and CD8.

Measurement of soluble CD4 and CD8 levels

The levels of soluble CD4 in blister fluid, horny-tissue extract and fractions from molecular sieve chromatography were assayed with an enzyme immunoassay using the non-competing murine anti-CD4 monoclonal antibodies (Cellfree CD4 Test Kit; T Cell Sciences, Cambridge, MA, U.S.A.) as described previously.[17] Briefly, 96-well microtitre plates were coated at 4°C overnight with the first monoclonal antibody against human CD4. The samples were added and incubated for 2 h at 37°C. The samples were then discarded, and each well received a horseradish peroxidase-conjugated monoclonal antibody that recognizes a different epitope on the CD8. After incubation with *o*-phenylenediamine for 30 min, the plates were read at 490 nm. Units of soluble CD4 were calculated from a standard curve constructed with serial dilutions of bovine-derived serum protein containing soluble CD4.

Soluble CD4 was assayed using a sandwich immunoassay with two monoclonal antibodies against CD8 antigen (T Cell Sciences) as previously described.[10,11,18] Following the binding of the first monoclonal antibodies to the wells of the microplate, the samples were then incubated for 90 min at 37°C followed by the removal of unreactive sample by washing. An enzyme-conjugated anti-CD8 monoclonal antibody directed against a second epitope on the CD8 molecule was then added and bound to the CD8 captured by the first antibody. Unbound enzyme-conjugated anti-CD8 was removed during a wash step and substrate solution added to the wells. The reaction was terminated by the addition of stop solution, and absorbance measured at 490 nm. A standard curve was prepared from five samples. The levels of soluble CD4 and CD8 in each of the samples were measured at the same time.

Protein measurement

The amount of protein in the horny-tissue extracts was measured using the Bio-Rad protein assay (Bio-Rad, Richmond, CA, U.S.A.).

Statistical analysis

Levels of soluble CD4 and CD8 were compared using the Wilcoxon rank-sum test.

Results

Soluble CD4 and CD8 in suction-blister fluid

The level of soluble CD4 in the suction-blister fluid from the involved skin in psoriasis was significantly higher (mean 19·1 ± 7·4 U/ml) than that found in the fluid from normal skin (12·5 ± 3·5 U/ml, $P<0{\cdot}05$) and the uninvolved skin in psoriasis (11·8 ± 2/5 U/ml, $P<0{\cdot}05$) (Fig. 1a). The level of soluble CD4 antigen in the suction-blister fluid obtained from the uninvolved skin of psoriasis was not significantly different from that of normal skin. The level of soluble CD8 in the suction-blister fluid in the involved skin of psoriasis (mean 149·7 ± 100·0 U/ml) was significantly higher than that obtained from normal skin (66·1 ± 14·0 U/ml, $P<0{\cdot}01$) and from the uninvolved skin of psoriasis (75·0 ± 28·2

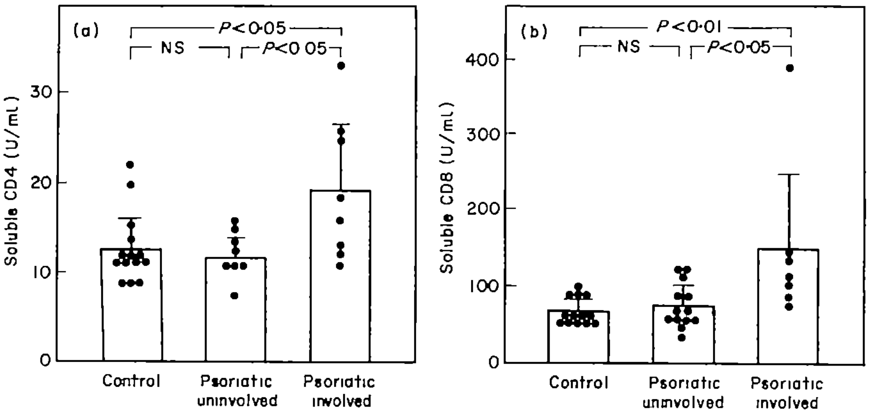

Figure 1. Levels of soluble CD4 (a) and CD8 (b) in the suction-blister fluids from control, psoriatic uninvolved and involved skin. Results are presented as means ± SD. NS, not significant.

U/ml, $P<0{\cdot}05$) (Fig. 1b). The soluble CD8 level in the suction-blister fluid from both the uninvolved skin in psoriasis and from normal skin were not significantly different. The protein content of the fluid obtained from suction blisters on normal skin and from the uninvolved and involved skin in psoriasis were not significantly different (19·3+2·7, 17·8±4·9 and 15·7±4·2 mg/ml, respectively). There was a significant relationship between the levels of CD4 and CD8 in the suction-blister fluids ($R=0{\cdot}48$, $P<0{\cdot}05$) (Fig. 2).

Soluble CD4 and CD8 in extracts of horny tissue

When the levels of soluble CD4 were expressed per ml horny-tissue extract, those in the horny-tissue extracts of psoriasis vulgaris (mean, 14·4±4·2 U/ml), pustular psoriasis (16·3±5·1 U/ml) and PPP (10·7±1·1 U/ml) were significantly higher than those of non-psoriatic hyperkeratotic skin (8·4±2·6 U/ml, $P<0{\cdot}5$, $P<0{\cdot}01$ and $P<0{\cdot}05$, respectively; Fig. 3a). The levels of soluble CD4 in the horny-tissue extracts of erythrodermic psoriasis (10·1±1·5 U/ml) were not significantly higher than those of non-inflammatory skin. The levels of soluble CD8 in the horny-tissue extracts of psoriasis vulgaris (mean, 42·4±31·2 U/ml) and pustular psoriasis (24·7±3·4 U/ml) were also significantly higher than those of non-psoriatic hyperkeratotic skin (19·1±0·8 U/ml, $P<0{\cdot}01$; Fig. 3b). The levels of soluble CD8 in the horny-tissue extracts of erythrodermic psoriasis (mean, 20·7 U/ml) and PPP (20·2±1·8 U/ml) were not significantly different from those found in non-inflamed skin.

Because the amounts of soluble proteins from the orthokeratotic horny samples were small, a significant difference in the levels of both CD4 and CD8 was found compared to psoriatic scales when they were expressed as per mg protein (data not shown).

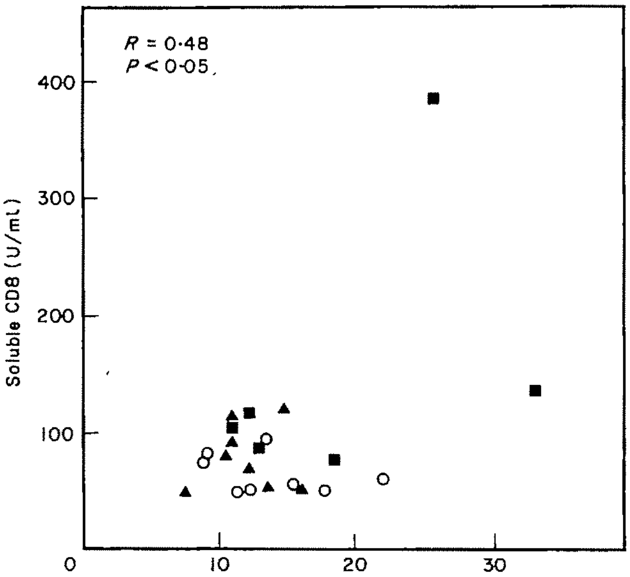

Figure 2. Relationship between the levels of soluble CD4 and CD8 in the suction-blister fluids from normal (○), psoriatic uninvolved (▲) and involved skin (■).

Molecular sieve chromatography

Gel chromatography was performed on two representative samples showing high levels of CD4 and CD8. Gel chromatography fractionation of suction-blister fluid with a high level of CD4 and of a psoriatic scale extract with a high level of soluble CD8 showed a peak at a M_r of 54 kDa (Fig. 4).

Discussion

Helper and suppressor T cells are considered to be involved in the pathogenesis of psoriasis[2,3,19] and increased levels of the soluble form of interleukin 2 receptor (IL-2R) suggest a role for activated T lympho-

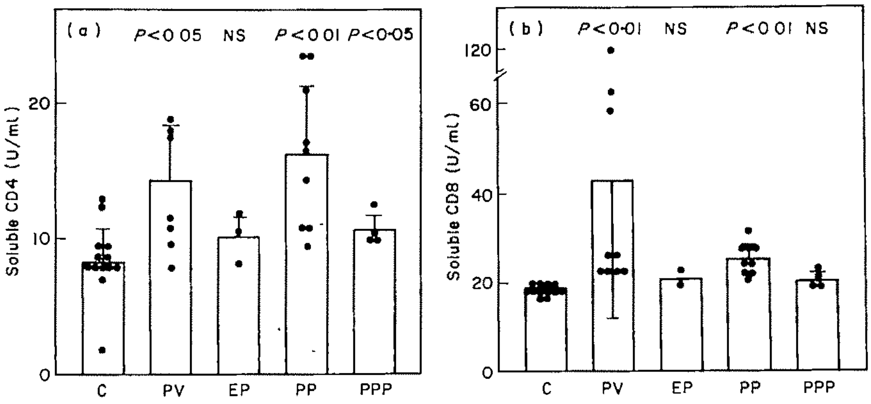

Figure 3. Levels of soluble CD4 (a) and CD8 (b) expressed per ml in the horny-tissue extracts of non-psoriatic hyperkeratotic skin (C), psoriasis vulgaris (PV), erythrodermic psoriasis (EP), pustular psoriasis (PP) and pustulosis palmaris et plantaris (PPP). Results are presented as means±SD.

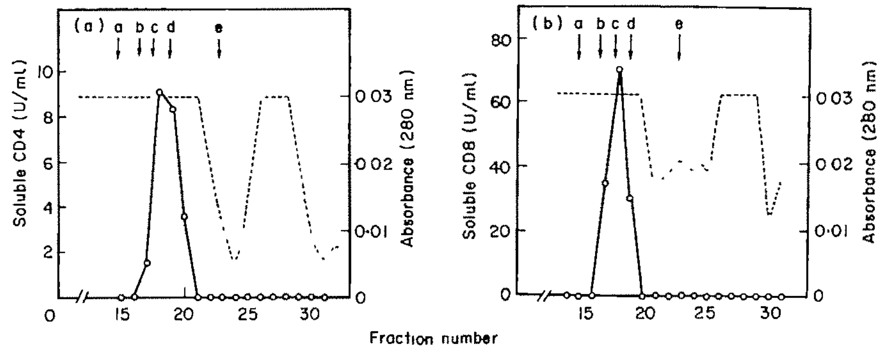

Figure 4. Molecular sieve chromatography of psoriatic-scale extract. A suction-blister fluid with a high level of soluble CD4 or a psoriatic-scale extract with a high level of soluble CD8 was loaded onto a TSKgel G2000SW gel-filtration column (7·5 × 30 cm). Fraction size was 0·5 ml, and flow rate was 0·5 ml/min. The protein content was estimated by the measurement of absorbance at 280 nm. Fractions were assayed for soluble CD4 (a) and CD8 (b) levels. The column was calibrated with blue dextran (void volume, a), γ-globulin (158 kDa, b, bovine serum albumin (67 kDa, c), ovalbumin (44 kDa, d) and carbonic anhydrase (29 kDa, e).

cytes.[16] The present study was designed to obtain direct evidence of the involvement of $CD4^+$ and $CD8^+$ lymphocytes in the lesions of psoriasis. An increase in the levels of both soluble CD4 and CD8 in the suction-blister fluid obtained from lesional skin strongly suggests an active role for these molecules and for helper and suppressor T cells in psoriasis. The increased levels of soluble CD4 and CD8 agree with the immunohistochemical findings that the dermal infiltrate in psoriasis consists mainly of helper T cells with a smaller compartment of suppressor T cells.[19] In the epidermis in psoriasis there is an increase in the number of $HLA\text{-}DR^+$ dendritic cells with an influx of activated helper T cells, and resolution of the lesions coincides with increased infiltration and activation of suppressor T cells.[2,20]

Because some suction-blister fluid from psoriatic lesional skin tended to become contaminated with blood that may contain $CD4^+$ or $CD8^+$ T cells, we only used suction-blister fluid that had very little blood contamination in this study. However, there is the possibility of a minor contribution of released CD4 or CD8 antigens from the blood. Also, blisters cannot be raised at the peripheral inflamed portions without any involvement of the central areas that may be regressing. The blister fluids therefore contained released CD4 and CD8 antigens from both these areas. A significant relationship between the levels of soluble CD4 and CD8 suggests that an infiltration of helper T cells was closely associated with an accumulation of suppressor T cells.

The soluble form of IL-2R, that is increased in the lesional skin in psoriasis,[16] has been suggested as an important regulatory molecule in controlling T-cell function.[21] In contrast, it has not been fully understood whether the soluble CD4 and CD8 molecules merely reflect cellular activity or have an active role.

Suction-blister fluids are assumed to reflect ongoing inflammatory reactions in the lesional skin, and extracts of horny tissue are considered to reflect the preceding events that have occurred in the epidermis.[16,22–24] In the present study, however, the levels of soluble CD4 and CD8 in the horny-tissue extracts obtained from the lesions of psoriasis vulgaris and pustular psoriasis were significantly higher than those obtained from non-psoriatic hyperkeratotic skin when expressed per ml of horny-tissue extract. Because of the high protein yields of the scale from the inflammatory skin lesions that contained exuded serum, the concentrations of CD4 and CD8 molecules in psoriasis and related dermatoses were very low when they were expressed as per mg of protein.

The increase in levels of soluble CD4 and CD8 and IL-2R in the suction-blister fluids from the involved skin in psoriasis suggests that the blister fluid was more representative of these inflammatory cell-derived molecules from the dermis, while inflammatory mediators released from epidermal keratinocytes accumulated in the horny tissue. We have also reported that the complement system is activated during suction-blister formation.[25]

Gel-column fractionation of a suction-blister fluid showed a 54-kDa peak for CD4, and that of a psoriatic scale extract showed a peak for CD8 with M_r of 54 kDa, which is 10 kDa smaller than the monodimeric CD8 molecule on the cell surface. It is presumed that there is a proteolytic cleavage of the membrane-bound molecules.[26] Our M_r determination indicated that the soluble CD8 molecules existed predominantly as a homodimer, and a little smaller than the membrane-associated form found in the lesional skin of psoriasis.

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References

1 Gottlieb AB. Immunologic mechanisms in psoriasis. *J Am Acad Dermatol* 1988; **18:** 1376–80.

2 Baker BS, Swain AF, Fry L, Valdimarsson H. Epidermal T Lymphocytes and HLA-DR expression in psoriasis. *Br J Dermatol* 1984; **110:** 555–64.

3 Hammar H, Gu S-Q, Johannesson A *et al.* Subpopulations of mononuclear cells in microscopic lesions of psoriatic patients: selective accumulation of suppressor/cytotoxic T cells in epidermis during the evolution of the lesion. *J Invest Dermatol* 1984; **83:** 416–20.

4 Lee RE, Gaspari AA, Lotze MT *et al* Interleukin 2 and psoriasis. *Arch Dermatol* 1988; **124:** 1811–15.

5 Ellis CN, Gorsulowsky DC, Hamilton TA *et al.* Cyclosporine improves psoriasis in a double-blind study. *JAMA* 1986; **256:** 3110–16.

6 Dalgleish AG. The T4 molecule: function and structure. *Immunol Today* 1986; **7:** 142–4.

7 Gay D, Maddon P, Sekaly R *et al.* Functional interaction between human T-cell protein CD4 and the major histocompatibility complex HLA-DR antigen. *Nature* 1987; **328:** 626–9.

8 Bushkin Y, Demaria S, Le J, Schwab R. Physical association between the CD8 and HLA class I molecules on the surface of activated human T lymphocytes. *Proc Natl Acad Sci USA* 1988; **85:** 3985–9.

9 Takada S, Engleman EG. Evidence for an association between CD8 molecules and the T cell receptor complex on cytotoxic T cells. *J Immunol* 1987; **139:** 3231–5.

10 Pui C-H, Ip SH, Dodge RK *et al.* Serum levels of CD8 antigen in childhood malignancies: a possible indicator of increased suppressor cell activity in poor-risk patients. *Blood* 1988; **72:** 1015–21.

11 Tomkinson BE, Brown MC, Ip SH *et al.* Soluble CD8 during T cell activation. *J Immunol* 1989; **142:** 2230–6.

12 Fisher RA, Bertonis JM, Meier W *et al.* HIV infection is blocked *in vivo* by recombinant soluble CD4. *Nature* 1988; **331:** 76–8.

13 Deen KC, McDougal JS, Inacker R *et al.* A soluble form of CD4 (T4) protein inhibits AIDS virus infection. *Nature* 1988; **331:** 82–4.

14 Fujimoto J, Levy S, Levy R. Spontaneous release of the Leu-2 (T8) molecule from human T cells. *J Exp Med* 1983; **158:** 752–66.

15 Kapp A. Elevated levels of soluble CD8 antigen in sera of patients with psoriasis: a possible sign of suppressor/cytotoxic T-cell activation. *Arch Dermatol Res* 1990; **282:** 6–7.

16 Takematsu H, Tagami H. Interleukin 2, soluble interleukin 2 receptor and interferon-γ in the suction blister fluids from psoriatic skin. *Arch Dermatol Res* 1990; **282:** 149–52.

17 Kline S, Carrabis S, Murphy K *et al.* Detection of a normal soluble CD4 level in human serum. *4th Annual Conference on Clinical Immunology and CIS Associated Meeting.* Arlington, VA, USA, 1989.

18 Symons JA, Wood NC, Di Giovine FS *et al.* Soluble CD8 in patients with rheumatic diseases. *Clin Exp Immunol* 1990; **80:** 354–9.

19 Bos JD, Hulsebosch HJ, Krieg SR *et al.* Immunocompetent cells in psoriasis: *in situ* immunophenotyping by monoclonal antibodies. *Arch Dermatol Res* 1983; **275:** 181–9.

20 Terui T, Aiba S, Kato T *et al.* HLA-DR antigen expression on keratinocytes in highly inflamed parts of psoriatic lesions. *Br J Dermatol* 1987; **116:** 87–93.

21 Robb RJ, Kutny RM. Structure–function relationships for the IL2-receptor system. IV. Analysis of the sequence and ligand-binding properties of soluble Tac protein. *J Immunol* 1986; **139:** 855–62.

22 Kobza Black A, Greaves MW, Hensby CN *et al.* A new method for recovery of exudates from normal and inflamed human skin. *Clin Exp Dermatol* 1977; **2:** 209–16.

23 Takematsu H, Tagami H. Granulocyte-macrophage colony-stimulating factor in psoriasis. *Dermatologica* 1990; **181:** 16–20.

24 Takematsu H, Ohmoto Y, Tagami H. Interleukin 1α and β in psoriatic lesional skin. *Tohoku J Exp Med* 1990; **161:** 159–60.

25 Ohkohchi K, Takematsu H, Tagami H. Determination of anaphylatoxin concentrations in suction blisters in patients with psoriasis. *J Invest Dermatol* 1986; **87:** 65–7.

26 Snow PM, Terhorst C. The T8 antigen is a multimeric complex of two distinct subunits on human thymocytes but consists of homomultimeric forms on peripheral blood T lymphocytes. *J Biol Chem* 1983; **258:** 14675–81.

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Superoxide dismutase in psoriasis, squamous cell carcinoma and basal cell epithelioma: an immunohistochemical study

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Summary Monoclonal antibodies against human Cu,Zn-superoxide dismutase (SOD) and Mn-SOD were used to stain frozen sections of normal and abnormal human skin. In normal human epidermis, the Cu,Zn-SOD antibody almost exclusively stained the basal cells. Mn-SOD antibody weakly stained the whole of the epidermis but more predominantly the basal cell layer. In psoriasis, Cu,Zn-SOD antibody mainly stained the basal cells of the lowest parts of the elongated rete ridges. Basal cells corresponding to the tip of the dermal papillae were weakly stained. Mn-SOD staining was considerably decreased in the psoriatic epidermis. In squamous cell carcinoma, staining with both Cu,Zn-SOD and Mn-SOD antibodies was decreased, and single cells positive for Cu,Zn-SOD were scattered throughout the tumour nests. In basal cell epithelioma, Cu,Zn-SOD staining was intense and diffusely distributed throughout the tumour nests, while Mn-SOD staining was absent.

Superoxide dismutase (SOD) catalyses the dismutation of the superoxide anion into O_2 and H_2O_2, thereby protecting cells against reactive free radicals by scavenging the superoxide radicals produced with ionizing radiation or through other mechanisms.[1]

In eukaryotic cells, there are two distinct types of SODs.[1,2] The predominant SOD is a soluble cytosolic enzyme containing copper and zinc at the catalytic site (Cu,Zn-SOD). The second type is the manganese-containing SOD (Mn-SOD) found in the matrix of the mitochondria. The Mn-SOD differs substantially from the Cu,Zn-SOD in primary structure.[3] Cu,Zn-SOD is inhibited by cyanides, whereas Mn-SOD is not.[1,2] Although extracellular SOD, which is similar to Cu,Zn-SOD, has been reported,[4] the physiological significance of this type of SOD remains unknown.

Recent evidence indicates decreased SOD activity in various cancer tissues.[5–8] Oberley and Buettner[5] suggested that the mitochondrial Mn-SOD is specifically decreased but numerous exceptions have since been reported.[9] Our previous studies have indicated that human lung cancer[10] and epithelial ovarian cancer[11] had increased levels of Mn-SOD. In the epidermis SOD activity is decreased in various hyperproliferative keratinocytes,[12–14] including squamous cell carcinoma (SCC), basal cell epithelioma (BCE) and in benign hyperproliferative skin such as psoriatic epidermis. Although decreased SOD activity is also observed in experimentally induced hyperproliferative epidermis, where Cu,Zn-SOD is specifically decreased,[12] the expression of the two types of SOD in various skin tumours or in psoriasis has remained unknown.

Polyclonal and monoclonal antibodies against Cu,Zn-SOD have been used to investigate the distribution of the enzyme in various tissues.[15,16] No information is available regarding the distribution of Cu,Zn-SOD in human skin. Recently one of us has produced a monoclonal antibody against human Mn-SOD[17] and with the two types of antibodies, differential staining of Cu,Zn-, and Mn-SOD is now possible. In the present study, we used these antibodies to investigate the distribution of SODs in normal skin and in various skin disorders.

Methods

Materials

The characterization of monoclonal antibodies specific to Cu,Zn-SOD and Mn-SOD has been previously described.[16,17] The Vectastatin ABC kit and biotinylated goat anti-mouse IgG were obtained from Vector (Burlingame, CA, U.S.A.) and the OCT compound from Miles Laboratory (Naperville, IL, U.S.A.). Normal mouse IgG

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Table 1. Summary of cases

	Case	Age	Sex	Biopsy sites
Normal	1	32	M	Back
	2	68	F	Chest
	3	76	M	Chest
	4	66	M	Axilla
	5	32	F	Thigh
Psoriasis	1	33	M	Back
	2	40	M	Thigh
	3	62	F	Buttock
	4	23	M	Elbow
	5	13	F	Chest
SCC	1	75	M	Thigh
	2	56	M	Groin
	3	88	F	Chest
BCE	1	77	F	Nose
	2	65	M	Nose
	3	70	M	Cheek

was obtained from Sigma (St Louis, MO, U.S.A.). All other reagents were of analytical grade.

Skin specimens

Normal skin specimens were obtained from patients undergoing elective plastic surgery. Skin biopsies were obtained from involved and uninvolved skin, before starting any therapy from five patients with psoriasis vulgaris, three patients with squamous cell carcinoma, and three patients with basal cell epithelioma (Table 1). Tissue samples were snap frozen in liquid nitrogen and stored at −80°C until used.

Immunohistochemical staining

Frozen sections (6 μm) of skin were air-dried and hydrated in phosphate-buffered saline (PBS). The sections were incubated with anti-Cu,Zn-SOD antibody (mouse ascitic fluid, diluted 1:400 in PBS) for 30 min or with anti-Mn-SOD antibody, PG 11, (mouse ascitic fluid, diluted 1:1000 in PBS) for 12 h. The avidin–biotin–peroxidase complex (ABC) technique was then used. The sections were washed in PBS, incubated with biotinylated goat anti-mouse antibody (diluted 1:100 in PBS) for 30 min, washed in PBS, and incubated with avidin–biotin complex (diluted 1:100 in PBS). The sections were then washed in PBS and incubated with aminoethylcarbazol in 0·02 M Na-acetate buffer (pH 5·0) for 10 min. Normal mouse IgG was used as a negative control. Staining with Masson–Fontana melanin was also performed on several specimens.

Results

Normal epidermis

Staining of Cu,Zn-SOD was located almost exclusively in the cytoplasm of the cells in the basal layer of the epidermis (Fig. 1a). Staining of the upper layers of the epidermis was negligible. Staining for Mn-SOD occurred throughout the epidermis but was more intense in the basal cell layer (Fig. 1b). Staining with anti-Mn-SOD antibody was weaker than with anti-Cu,Zn-SOD antibody and a longer incubation time was usually required.

Psoriatic epidermis

The distribution of Cu,Zn-SOD and Mn-SOD in the uninvolved epidermis of psoriatic patients was similar to that in the normal epidermis.

In the involved epidermis, Cu,Zn-SOD was located mainly in the basal cells (Fig. 2a). Several cell layers

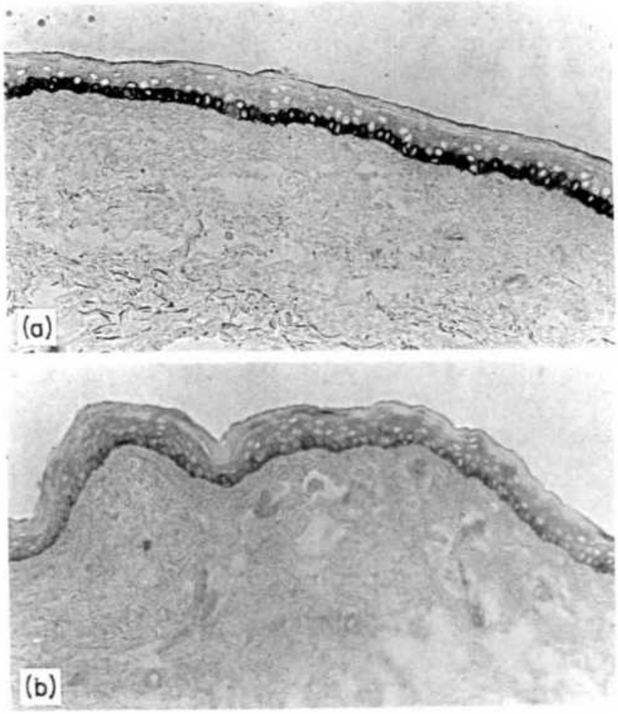

Figure 1. Staining of Cu,Zn-SOD and Mn-SOD in normal skin. (a) Cu,Zn-SOD staining of normal epidermis. Only the basal cells are intensely stained (× 242). (b) Mn-SOD staining of normal epidermis. The whole epidermis is weakly stained with stronger staining of the basal cell layer (× 242).

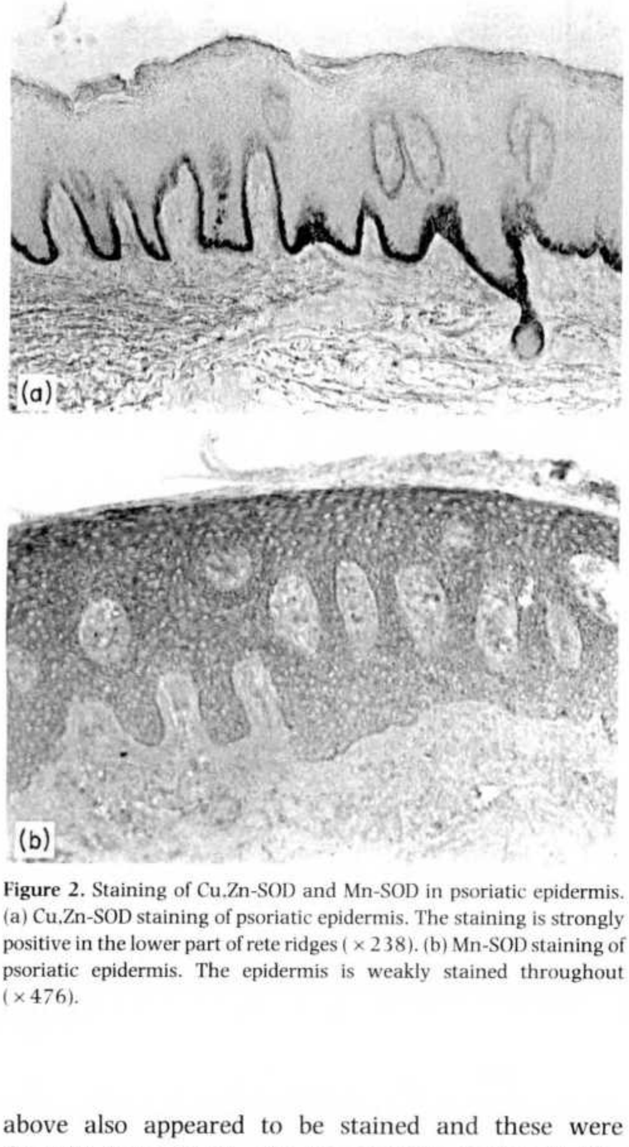

Figure 2. Staining of Cu,Zn-SOD and Mn-SOD in psoriatic epidermis. (a) Cu,Zn-SOD staining of psoriatic epidermis. The staining is strongly positive in the lower part of rete ridges (× 238). (b) Mn-SOD staining of psoriatic epidermis. The epidermis is weakly stained throughout (× 476).

above also appeared to be stained and these were thought to be basal cells tangentially sectioned. The staining was strongly positive in the lower part of rete ridges while the basal cell layer corresponding to the tip of the dermal papillae was weakly stained (Fig. 2a).

The psoriatic involved epidermis stained weakly throughout with anti-Mn-SOD antibody (Fig. 2b). Minimal staining was observed even in the basal cell layer.

Skin tumours

In SCC, staining with both Cu,Zn-SOD antibody and Mn-SOD antibody was considerably decreased (Fig. 3). Many peripheral cells corresponding to the basal cell layer were only weakly stained or not at all with the anti-Cu,Zn-monoclonal antibody (Fig. 3a, b). An irregular staining pattern consisting of single cells positive for

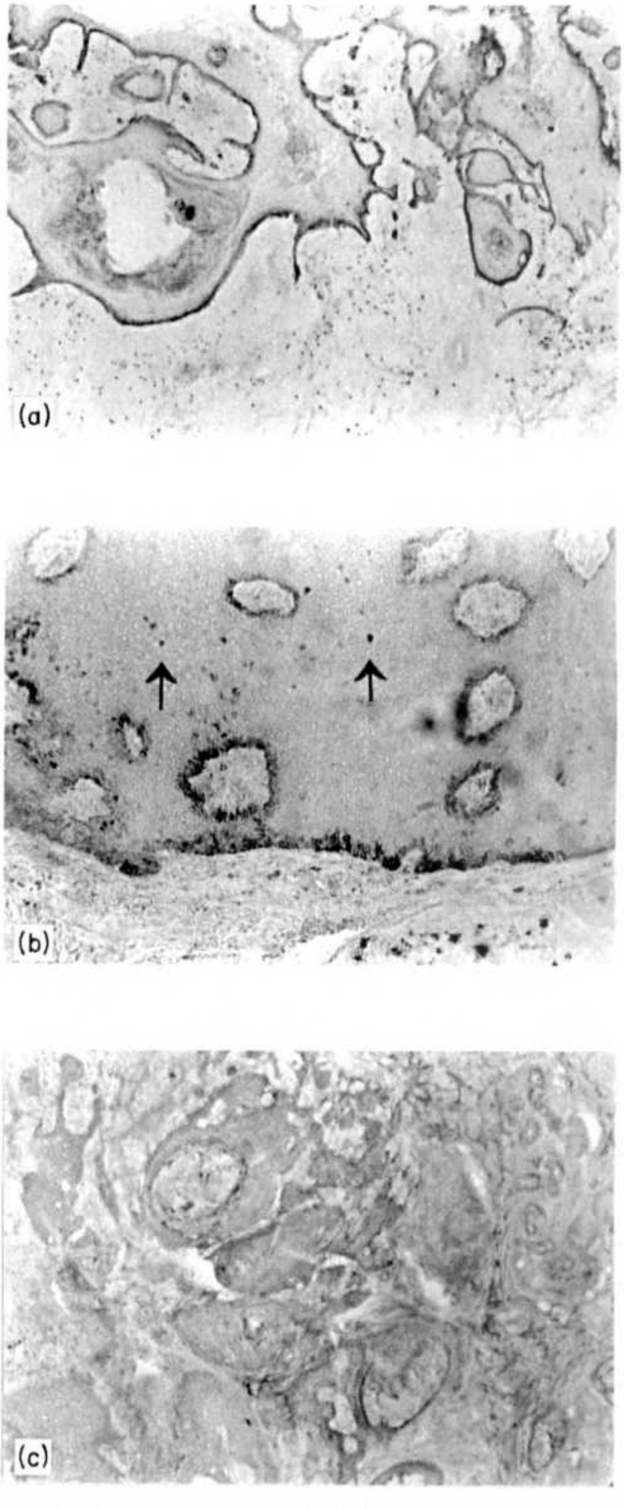

Figure 3. Staining of Cu,Zn-SOD and Mn-SOD in squamous cell carcinoma. (a) Cu,Zn-SOD staining of squamous cell carcinoma. The staining is relatively weak and irregularly distributed (× 238). (b) Cu,Zn-SOD staining of squamous cell carcinoma. Irregular staining of the outermost cells is observed, with single positive cells (arrows) in the tumour nests (× 476). (c) Mn-SOD staining of squamous cell carcinoma. The tumour nests are only weakly stained (× 238).

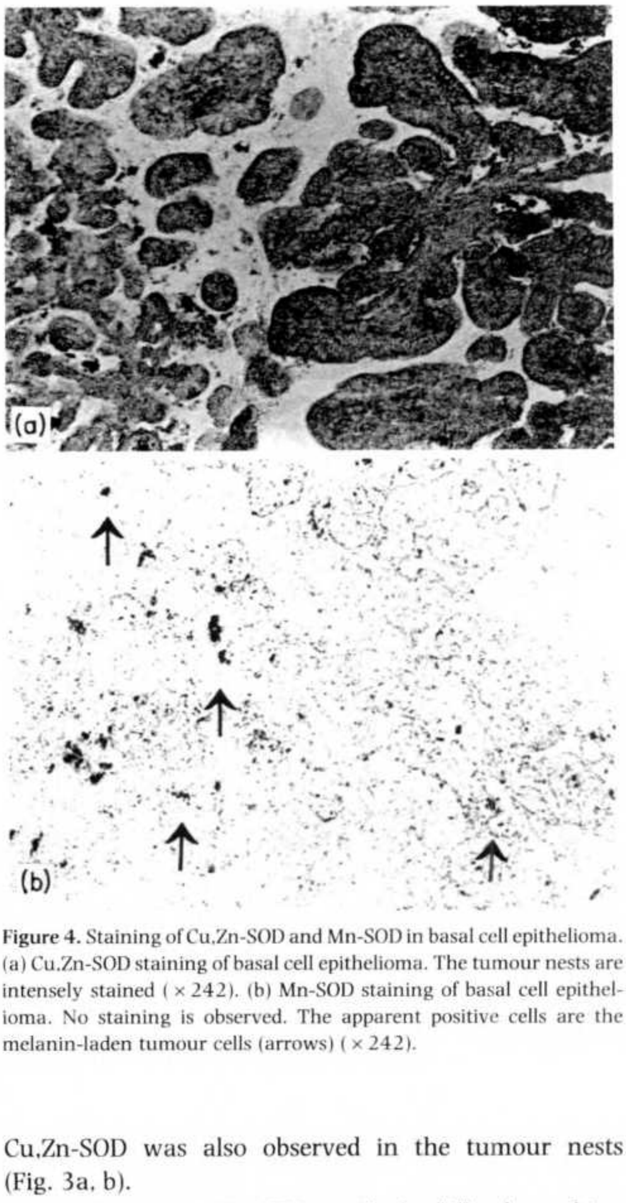

Figure 4. Staining of Cu,Zn-SOD and Mn-SOD in basal cell epithelioma. (a) Cu,Zn-SOD staining of basal cell epithelioma. The tumour nests are intensely stained (× 242). (b) Mn-SOD staining of basal cell epithelioma. No staining is observed. The apparent positive cells are the melanin-laden tumour cells (arrows) (× 242).

Cu,Zn-SOD was also observed in the tumour nests (Fig. 3a, b).

In BCE, anti-Cu,Zn-SOD antibody diffusely and intensely stained the whole of the tumour nests (Fig. 4a) but Mn-SOD staining was absent (Fig. 4b). Cells that appeared positive were melanin-laden tumour cells as confirmed by melanin staining.

The epidermis adjacent to SCC or BCE showed no abnormal staining pattern and the distribution of Cu,Zn-SOD and Mn-SOD was similar to that in the normal epidermis.

Discussion

To our knowledge this is the first report of the differential staining for each type of SOD in human skin and in various disorders. Although the immunoreactive SOD might not necessarily be associated with enzyme activity,[10] our results indicate that the normal human epidermis contains both Cu,Zn-SOD and Mn-SOD, which appear to be differently affected in various skin disorders.

Recently, using electron spin resonance, Ogura *et al.*[18] reported that SOD activity in cow snout epidermis was distributed throughout the epidermis, with more intense activity in the lower layer than the upper and middle layers. Their results agree with ours, although they apparently assume that the epidermal SOD is of the Cu,Zn-type.[18] This would probably reflect the weak activity of Mn-SOD in normal epidermis,[2,12,19] and we found that the staining of Mn-SOD was much weaker than that of Cu,Zn-SOD in our study.

In psoriasis skin, Cu,Zn-SOD was mainly located in the lowest part of the elongated rete ridges, which are the most proliferative sites in this disorder.[19] It has been reported that the SOD activity is markedly decreased in the hyperproliferative psoriatic epidermis.[12,14] The decreased SOD activity might reflect decreased levels of Cu,Zn-SOD in basal cells corresponding to the tip of dermal papillae and/or decreased levels of Mn-SOD in the involved psoriatic epidermis.

In SCC, although not distributed regularly as in the psoriatic epidermis, the staining of both Cu,Zn-SOD and Mn-SOD was decreased, which again agrees with findings of decreased SOD activity in SCC.[12,13] We also found a disorganized pattern showing single cells positive for SOD in the tumour nests. A similar pattern has been described in SCC using antibodies which detect specific keratinocyte antigens.[20,21]

A marked difference from normal skin was observed in BCE, where staining for Cu,Zn-SOD was diffuse and intense. The staining was so marked that all the tumour cells resembled normal basal cells, a finding that is occasionally observed in this disorder.[22-24] Mn-SOD, which is also predominantly found in the normal basal cells, was however absent in BCE.

Recently several investigators[25-27] reported that Mn-SOD and Mn-SOD mRNA are induced by interleukin 1 (IL-1). The decreased staining for Mn-SOD in psoriasis and in BCE is interesting because IL-1 is reported to be decreased in these disorders.[28-30] However, in SCC an increase in IL-1 has been reported,[29] while we found a decrease in staining for Mn-SOD.

The role of SOD(s) in protecting cells against the toxicity of reactive oxygen species has been well established. Recent evidence suggests that SOD might also be associated with keratinocyte proliferation.[12,31] The role of SOD in inhibiting the oncogenic activity of X-rays and of tumour-promoting phorbol esters has been described.[32] The decreased SOD activity in various hyperproliferative skin disorders should have a significant effect, although the expression of SOD differs among the skin disorders.

Acknowledgments

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References

1 Fridovich I. The biology of oxygen radicals. *Science* 1978; **201**: 875–80.
2 Carraro C, Pathak MA. Characterization of superoxide dismutase from mammalian skin epidermis. *J Invest Dermatol* 1988; **90**: 31–6.
3 Steinman HM, Hill RL. Sequence homologies among bacterial and mitochondrial superoxide dismutase. *Proc Natl Acad Sci USA* 1973; **70**: 3725–9.
4 Marklund SL. Extracellular superoxide dismutase and other superoxide dismutase isoenzymes in tissues from nine mammalian species. *Biochem J* 1984; **222**: 649–55.
5 Oberley LW, Buettner GR. Role of superoxide dismutase in cancer: a review. *Cancer Res* 1979; **39**: 1141–9.
6 Marklund SL, Westman NG, Lundgren E, Roos G. Copper- and zinc-containing superoxide dismutase, manganese-containing superoxide dismutase, catalase, and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues. *Cancer Res* 1982; **42**: 1955–61.
7 Tisdale MJ, Mahmoud MB. Activities of free radical metabolizing enzymes in tumours. *Br J Cancer* 1983; **47**: 809–12.
8 Konstantinov AA, Peskin AV, Popova EY *et al.* Superoxide generation by the respiratory chain of tumor mitochondria. *Biochim Biophys Acta* 1987; **894**: 1–10.
9 Westman NG, Marklund SL. Copper- and zinc-containing superoxide dismutase and manganese-containing superoxide dismutase in human tissues and human malignant tumors. *Cancer Res 1981*: **41**: 2962–6.
10 Iizuka S, Taniguchi N, Makita A. Enzyme-linked immunosorbent assay for human manganese-containing superoxide dismutase and its content in lung cancer. *J Natl Cancer Inst* 1984; **72**: 1043–9.
11 Ishikawa M, Yaginuma Y, Hayashi H *et al.* Reactivity of a monoclonal antibody to manganese superoxide dismutase with human ovarian carcinoma. *Cancer Res* 1990; **50**: 2538–42.
12 Ohkuma N, Kajita S, Iizuka H. Superoxide dismutase in epidermis: its relation to keratinocyte proliferation. *J Dermatol (Tokyo)* 1987; **14**: 562–8.
13 Galeotti T, Borrello S, Seccia A *et al.* Superoxide dismutase content in human epidermis and squamous cell epithelioma. *Arch Dermatol Res* 1980; **267**: 83–6.
14 van Baar HMJ, van de Kerkhof PCM, Schalkwjk J, Mier PD. Cutaneous superoxide dismutase activity in psoriasis. *Br J Dermatol* 1987; **116**: 462.
15 Thaete LG, Crouch RK, Spicer SS. Immunolocalization of copper, zinc superoxide dismutase. II. Rat. *J Histochem Cytochem* 1985; **33**: 803–8.
16 Oka S, Ogino K, Matsuura S *et al.* Human serum immunoreactive cooper-, zinc-superoxide dismutase assayed with an enzyme monoclonal immunosorbent in patients with digestive cancer. *Clin Chim Acta* 1989; **182**: 209–20.
17 Kawaguchi T, Noji S, Uda T *et al.* A monoclonal antibody against COOH-terminal peptide of human liver manganese superoxide dismutase. *J Biol Chem* 1989; **246**: 5762–7.
18 Ogura R, Ueta H, Sugiyama M, Hidaka T. Distribution of superoxide dismutase activity in the epidermis: Measurement with electron spin resonance spin trapping. *J Invest Dermatol* 1990; **94**: 227–9.
19 Ohkuma N, Matsuo S, Iizuka H, Ohkawara A. Superoxide dismutase in epidermis. *J Dermatol (Tokyo)* 1987; **14**: 218–23.
20 Van Neste DJJ, Staquet MJ, Leroy BP, De Coster WJ. Distribution pattern of psoriatic keratoblasts: computer-assisted image-analysis and expression of 67 kD keratin polypeptides in the epidermis of stable plaques of psoriasis. *J Invest Dermatol* 1988; **90**: 382–6.
21 Weiss RA, Guillet GYA, Freedberg IM *et al.* The use of monoclonal antibody to keratin in human epidermal disease: alterations in immunohistochemical staining pattern. *J Invest Dermatol* 1983; **81**: 224–30.
22 Said JW, Sassoon AF, Shintaku IP, Banks-Schlegel S. Involucrin in squamous and basal cell carcinoma of the skin: an immunohistochemical study. *J Invest Dermatol* 1984; **82**: 449–52.
23 Eto H, Hashimoto K, Kobayashi H *et al.* Monoclonal antikeratin antibody: production, characterization, and immuno-histochemical application. *J Invest Dermatol* 1985; **84**: 404–9.
24 Habets JMW, Tank B, Vuzevski VD *et al.* The detection of basal cell determinants in human basal cell carcinomas using two different monoclonal antibodies. *Acta Derm Venereol (Stockh)* 1987; **67**: 391–8.
25 Wong GHW, Goeddel DV. Induction of manganese superoxide dismutase by tumor necrosis factor: possible protective mechanism. *Science* 1988; **242**: 941–4.
26 Masuda A, Longo DL, Kobayashi Y *et al.* Induction of mitochondrial manganese superoxide dismutase by interleukin 1. *FASEB J* 1988; **2**: 3087–91.
27 Visner GA, Dougall WC, Wilson JM *et al.* Regulation of manganese superoxide dismutase by lipopolysaccharide, interleukin-1, and tumor necrosis factor. *J Biol Chem* 1990; **265**: 2856–64.
28 Takematsu H, Suzuki R, Tagami H, Kumagai K. Interleukin-1-like activity in horny layer extracts: Decreased activity in scale extracts of psoriasis and sterile pustular dermatosis. *Dermatologica* 1986; **172**: 236–40.
29 Mizoguchi M, Kawa Y, Furusawa Y *et al.* Interleukin 1 alpha and beta in squamous cell carcinoma and basal cell epithelioma. *J Invest Dermatol* 1989; **92**: 483.
30 Hammerberg C, Fisher G, Baadsgaard O *et al.* Characterization of IL-1 and inhibitor in psoriatic and normal skin. *J Invest Dermatol* 1989; **92**: 439.
31 Solanki V, Rana R, Slaga T. Diminution of mouse epidermal superoxide dismutase and catalase activities by tumor promoters. *Carcinogenesis* 1981; **2**: 1141–6.
32 Borek C, Troll W. Modifiers of free radicals inhibit *in vitro* the oncogenic actions of X-rays, bleomycin, and tumor promoter 12-o-tetradecanoylphorbol 13-acetate. *Proc Natl Acad Sci USA* 1983; **80**: 1304–7.

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Malignant melanoma occurring in those aged under 30 in the west of Scotland 1979–1986: a study of incidence, clinical features, pathological features and survival

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Summary

The study population consisted of the 95 patients who presented with cutaneous malignant melanoma in the west of Scotland between 1979 and 1986 and who were aged under 30 at the time of diagnosis. Over this 7-year period, 1299 melanomas were diagnosed in all age groups in this geographical area, increasing in incidence by 82% from 135 in 1979 to 246 in 1986. The proportion of melanomas diagnosed before the patients' thirtieth birthday remained constant at 6%. None were diagnosed in the under-15 age group, and none developed on giant congenital naevi. Forty-two of the 95 melanomas (44%) in the under-30 group developed on a small naevus present either from birth or early childhood. These melanomas were thicker than those apparently developing on normal skin, but once controlled for tumour thickness and sex the presence of a pre-existing naevus did not affect 5-year survival. The overall 5-year survival was 76% with poorer survival associated with thicker tumours and male sex.

This study suggests that small early onset naevi may have a higher potential for post-pubertal malignant change than has been previously recognized.

Malignant melanoma is a solid tumour which affects adults in all age groups with a reported world-wide average age at diagnosis of 45 years.[1] Children are rarely affected. In the early 1980s it appeared that the proportion of patients in the west of Scotland presenting with melanoma in the second and third decade of life was rising. Because of this, and because of the fact that in some other tumour systems such as cervical carcinoma the tumour may be more aggressive in younger people,[2] we carried out a study of melanoma in this age group to establish whether or not there was a disproportionate increase in melanoma in this age group, to identify any distinguishing clinical or histological features in these younger patients, and also to determine 5-year survival figures for this section of the melanoma population.

Methods

Patients

The study population consisted of all the patients presenting in the west of Scotland in the years 1979–86 inclusive with primary cutaneous malignant melanoma diagnosed before their thirtieth birthday. Data on patients was drawn from the records of the West of Scotland section of the Scottish Melanoma Group which aims to register all patients presenting with invasive primary cutaneous melanoma in Scotland. Patients are first registered by the pathologist who reports the case, and full pathological details including information on prognostic variables are sent to the local Scottish Melanoma Group co-ordinator.[3] Pathological details include the histogenetic type, evidence of pre-existing naevus, level of invasion, tumour thickness mitotic rate, evidence of regression, and gross and microscopic details of excision margins. Surgical details are also recorded, and annual follow-up information sought. Cross checks of completeness of registration of cases are made with the central Cancer Registries, and with all hospitals in Scotland, both private and NHS.

Comparative sample

In the course of the study, it became clear that an age- and sex-matched comparative group was required to establish the prevalence of early onset naevi (EON),

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defined as melanocytic naevi present since birth or developing prior to the age of 2 years in this geographical area. This was because parents found it difficult to be certain that a naevus had been present at birth. All the comparative group were asked if they had any such naevi, and were then fully examined both to confirm the nature of lesions considered by the controls to be melanocytic naevi and also to identify any naevi not reported by the subject. A total of 161 subjects were identified, 83 women and 78 men with a mean age of 22·6 years. This comparative group was used only to obtain a figure for the prevalence of these naevi in the normal population.

Statistical analysis

The logistic regression model using stepwise variable selection was used for multivariate analysis to assess the joint effect of variables on 5-year survival.

Results

The numbers of patients first presenting in the west of Scotland with melanoma during the study period, and the numbers of these under 30 at time of diagnosis are recorded in Table 1. In common with the rest of Scotland and all other parts of the world for which figures are available, there was a steady rise in the number of patients with melanoma which increased by 111 from 135 in 1979 to 246 in 1986, a rise of 82% over the 8-year period or 10% per year. While the number of melanomas diagnosed in young patients also rose, the rate of increase was less and, apart from in 1983, the proportion remained relatively constant at 6% of the annual total. The ratio of females to males for the entire melanoma population was 2:1, and for the young patients it was similar at 61 females and 34 males. Throughout the 7-year period, case ascertainment was constant and complete for the whole of Scotland, and there was no significant change in the total number or age distribution of the population.

Table 1. Number of patients aged under 30 at diagnosis of primary melanoma in relation to total number of cutaneous melanomas

Year	Total no. melanomas	F	M	No. <30 years (% of total)	F	M
1979	135	99	36	9	7	2
1980	151	102	49	13	6	7
1981	139	93	46	9	6	3
1982	138	89	49	9	6	3
1983	169	114	55	19	10	9
1984	187	121	66	9	5	4
1985	209	141	68	14	11	3
1986	246	168	78	13	10	3
Total	1374	927	447	95 (6 9)	61 (6·6)	34 (7·6)

The age at diagnosis divided by sex is shown in Table 2. No patient aged 15 years or less developed melanoma, but there were three cases aged 15–19, 23 aged 20–24 and 69 aged 25–29 years at the time of diagnosis. The three patients aged 15–20 were all female and there were two lesions on the leg and one on the arm.

Forty-two of the young patients (44%) gave a history, frequently confirmed personally by a parent or by infant pictures, that an early onset melanocytic naevus (EON) that had been present since birth or developed prior to the age of 2 years had been present at the site on which their melanoma subsequently developed (Table 3). In 14 other cases (15%) the melanoma arose on a naevus present since the early teens, and in 39 patients (41%) there was no pre-existing pigmented lesion. Histological examination of melanomas from the 42 patients with a history and/or clinical features of an early onset naevus, showed that in 38 (91%) there were residual naevus cells in the lower part of the dermis or in close proximity to appendageal structures in sites consistent with a congenital type naevus. The size of these early onset naevi varied from 4 mm to >1 cm, but there were no giant naevi.

Table 2. Age at diagnosis

Age	F	M	Total
0–15	0	0	0
16–19	3	0	3
20–24	18	5	23
25–29	40	29	69
Total	61	34	95

Table 3. Presence of early onset or later acquired naevus

	F	M	Total
Early onset naevus present	25	17	42
Later acquired naevus	11	3	14
No naevus present	25	14	39
Total	61	34	95

Table 4. Site of melanoma divided by presence of early onset naevus, acquired naevus or no naevus

	Leg	Arm	Trunk	Head and neck	Sole of foot	Total
Early onset naevus	16	6	13	6	1	42
Later acquired naevus	8	2	1	1	2	14
No naevus	21	4	12	2	0	39
Total	45	12	26	9	3	95

The age-matched comparison group gave a history of an early onset melanocytic naevus, as defined above, in 32 of 161 subjects (20%). In all cases the presence of a naevus was confirmed by clinical examination. These lesions were observed with equal frequency in male and female controls.

The body site on which the melanoma developed is shown in Table 4. Forty-five of the lesions (47%) were on the lower limb, 26 on the trunk, 12 on the upper limb, nine on the head and neck and three on the sole of the foot. In females, the leg was the commonest site (30/61, 49%) and in men it was the trunk (13/34, 38%). In older patients, the leg is also the commonest site for females and the trunk for males. Lesions on the head and neck comprised 10% of the total, and form a lower proportion of all melanomas in the young group, compared with 20% in older patients.

Seventy-one (75%) of the melanomas in the young study group were of the superficial spreading type, 19 (20%) were nodular melanomas, five were acral lentiginous melanomas and there were no lentigo maligna melanomas (Table 5). In the comparative melanoma sample of all ages collected over the same time period, 48% of all lesions were of the superficial spreading type, 23% were nodular melanomas and 15% were lentigo maligna melanomas.

Fifty-seven (61%) of the melanomas in the young age group were <1·5 mm thick, 21 (22%) were 1·5–3·49 mm thick, and 17 (18%) were >3·5 mm thick (Table 6). In older patients over this time period only 40% of all lesions were in the thin category, so thin melanomas were more frequent in the young group. However there were some extremely thick lesions in the >3·5 mm category (e.g. one at 11 mm, two at 14 mm and one at 25 mm) in the under-30 age group. Three of these arose in EONs.

Survival

Five-year survival figures for the under-30 group show that 72 (74%) were alive and disease-free, 18 (20%) had died of melanoma, five were alive with melanoma, and no patient had died of other causes (Table 7). Preliminary analysis showed that the five patients who were alive with disease had very similar characteristics to those who were dead, and obviously the proportion of such patients at 5% is small. The detailed analysis therefore relates to those who were alive and disease-free compared with those who were dead or with recurrent disease considered together.

It would appear that patients whose melanomas arise on a congenital or early onset naevus have a slightly poorer prognosis, with only 69% being alive and tumour-free as against 79% for those whose melanoma was apparently not associated with a naevus and 86% for those whose melanoma had arisen in association with a later onset naevus. However, when the logistic regression model is applied, and adjustment made for tumour thickness, there is no significant association between any type of naevus and survival. This contrasts

Table 5. Histogenetic type of melanoma

	Superficial spreading	Nodular	Acral	Total
Early onset naevus	32	8	2	42
Later acquired naevus	10	1	3	14
No naevus	29	10	0	39
Total	71	19	5	95

Table 6. Breslow thickness (mm) of primary tumour

	0–1·49	1·5–3·49	3·50+	Total
Early onset naevus	22	10	10	42
Later acquired naevus	12	2	0	14
No naevus	23	9	7	39
Total	57	21	17	95

Table 7. Five-year survival (percentages in parentheses)

	Alive disease free			Alive with melanoma			Died of melanoma		
	All	Female	Male	All	Female	Male	All	Female	Male
Melanoma on early onset naevus	29 (69)	20 (80)	9 (53)	3	1	2	10	4	6
Melanoma on pubertal naevus	12 (86)	10 (91)	2 (67)	1	1	0	1	0	1
Melanoma with no naevus	31 (79)	22 (88)	9 (64)	1	0	1	7	3	4
Total	72 (74)	52 (85)	20 (59)	5	2	3	18	7	11

with the observation of Friedman *et al.*[4] who recorded a more favourable prognosis for patients whose melanomas arose in association with an acquired naevus. However the two studies are not comparable in that Friedman was specifically investigating acquired rather than congenital naevi, and included patients of all ages in his study. Measurement of Breslow thickness in cases such as those included in Friedman's series is notoriously difficult, as it involves differentiating the deepest naevus cell separately from the deepest tumour cell.

The two statistically significant findings related to survival were the Breslow thickness of the melanoma and, slightly less powerful, the sex of the patient with males carrying significantly poorer prospects for survival compared with females once tumour thickness was controlled for. Thus the poorer survival in the group associated with an EON is explained by the thicker tumours in this group. Other features studied in the logistic regression model included age, site, and histogenetic type, none of which were of prognostic significance when corrected for tumour thickness and sex, although histogenetic type was by itself significant, with nodular melanomas showing a poorer 5-year survival than the superficial spreading type.

Discussion

The initial clinical impression that the proportion of young patients presenting with melanoma was increasing has not been confirmed. In this group of patients our data shows that the rate of increase in melanoma incidence is less than in the older age group. The 7-year period of observation is however short for epidemiological observations, and our ongoing registration of all melanomas in Scotland will establish whether or not there is any change over a longer period of time. If confirmed, however, this would suggest that the factors which cause melanoma require a longer latent period to have an effect, and are not fully felt until after the third decade. The age distribution in our under-30 group, with a steady increase in numbers between 16 and 30, would support this view.

The fact that female sex was a good prognostic factor in this group of young patients suggests that the survival advantage for females noted in this and many other studies is a phenotypic rather than an acquired characteristic. There are no obvious differences between the two sexes as regards habits associated with the development of melanoma, such as sun exposure, in the first three decades of life.

The sex and site distribution of the melanomas in the under-30 group were similar to those in the older patients, other than lesions on the head and neck which were less frequent in the younger patients. The histogenetic types differed, with no lentigo maligna melanomas in the younger patients and a higher proportion of superficial spreading melanomas. At present it is believed that cumulative lifetime sun exposure is more important for the development of lentigo maligna melanomas than for other types, and our observations are consistent with this view. The higher proportion of thin melanomas in younger patients may reflect a greater awareness of the significance of pigmented lesions, and also greater concern for appearance.

An unexpected observation that emerged from this study is the high proportion of melanomas in young patients which arose on early onset naevi. No other group has yet studied a young population with melanoma and there are therefore no comparative studies to quote. Rhodes *et al.* in a pathological study of a series of 234 melanomas have also suggested that small congenital naevi may be biologically important precursors of melanoma.[5] In this series, six of 18 melanomas identified as arising on small congenital naevi were in patients who were under 30 at the time of diagnosis, but the age structure of the entire study population was not given. Our experience of questioning older patients about a long-standing pre-existing naevus is that memories are unreliable, and parental confirmation, an important part of our study on younger patients, is rarely available. Our observation requires confirmation but suggests that small congenital or early onset naevi may be a more common precursor to melanoma than has previously been recognized.

Because we included in our study naevi which had appeared in the first 2 years of life, as well as congenital naevi, an appropriate locally derived comparative group was required to assess the prevalence of such naevi in the normal population. Our results indicate that one-fifth of the population may have naevi in this category. This suggests that the frequency of malignant change in these naevi is relatively low and that removal of all such naevi is neither necessary nor practical. If, however, our results are confirmed, it would appear to be advisable to warn the public that naevi appearing early in life should be observed carefully with regard to growth or change after puberty.

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References

1 Balch CM, Milton GW. *Cutaneous Melanoma*, 1st edn, Lippincott: 1985, 510.
2 Hall SW, Monaghan JM. Invasive carcinoma of the cervix in younger women. *Lancet* 1983; ii: 731–3.
3 MacKie RM, Smyth JF, Soutar DS *et al.* Malignant melanoma in Scotland 1979–1983. *Lancet* 1985; ii: 859–62.
4 Friedman RJ, Rigel DR, Kopf AW *et al.* Favourable prognosis for malignant melanomas associated with acquired melanocytic naevi. *Arch Dermatol* 1983; 119: 455–62.
5 Rhodes AR, Sober AJ, Day CL *et al.* The malignant potential of small congenital naevi. *J Am Acad Dermatol* 1982; 6: 230–41.

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Measurement of involved surface area in patients with psoriasis

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Summary

Three methods of measuring the surface area of the involved skin were compared in 10 patients with psoriasis. Using the rule of nines, four untrained observers estimated the average extent of psoriasis as 20, 14, 23 and 33% of the body surface area. Measuring the area of tracings of plaque outlines using image analysis gave a mean involved surface area of 9% and image analysis of whole body photographs gave a mean value of 7%. We conclude that untrained observers using the rule of nines will overestimate the extent of psoriasis and that image analysis of whole body photographs is comparable to that of traced outlines.

The assessment of the effectiveness of new treatments for psoriasis is limited by the lack of any objective measure of disease severity. The currently used rule of nines method for assessing the total area of involvement forms a critical part of the PASI system[1] of assessing the severity of psoriasis and was originally developed for estimating the surface area of burns.[2] The rule assumes that the total body surface area comprises 9% for the head and neck, each arm, anterior and posterior leg, and the four trunk quadrants, leaving 1% for the genitalia. Previous studies have indicated wide variations between observers' estimates.[3] The extent of psoriasis is possibly the single most important factor in deciding treatment options and patient disability and could be accurately measured given developments in image analysis. We have studied the current rule of nines method of area assessment and have compared the results to two other objective methods in which areas were measured using a computer image analysing system.

Methods

Ten in-patients (five females, five males; mean age 31 years, range 17–65 years) with chronic plaque psoriasis were selected over a 9-month period. Five patients had large, confluent plaques and five had small plaque psoriasis. The percentage body surface area involved by psoriasis was assessed using the following three different methods.

Rule of nines

Four observers including three dermatologists and a dermatology nurse of 8, 4, 1 and 11 years' experience, respectively, were asked to assess the extent of psoriasis over two consecutive days using the rule of nines. The observers had no record of their previous estimations on the second day of observation and the order of assessment of the extent of the disease on both arms and legs and the front and back of the trunk were randomized to minimize memory recall bias. From these estimates of involved surface area, for each of the six body sites, a figure for the total percentage body area affected was calculated, excluding the 10% for head, neck and groin areas.

Plaque tracings

The plaque outlines for all body sites, excluding the head, neck and groin areas, were traced onto clear polythene sheets and the traced areas measured. In each patient six sheets were used to include the six body sites of both arms and legs and the anterior and posterior trunk; this process took approximately 60 min/patient. These tracings, including a 2-cm diameter circle for calibration, were reduced to 21 × 30 cm black-and-white photographs and the outlined plaque area was measured using a computer image analysis system. Before image analysis, the photographs of the plaque tracings had to be checked to ensure that there was a uniform thickness of traced outline with no breaks in the lines and very small areas had to be filled in, using a marker pen, to ensure that these were identified by the computer. Without these image adjustments, which took a further 60 min/patient, the image analysis system joined up adjacent plaques or merged multiple plaques, thus producing an inaccurate image for measurement.

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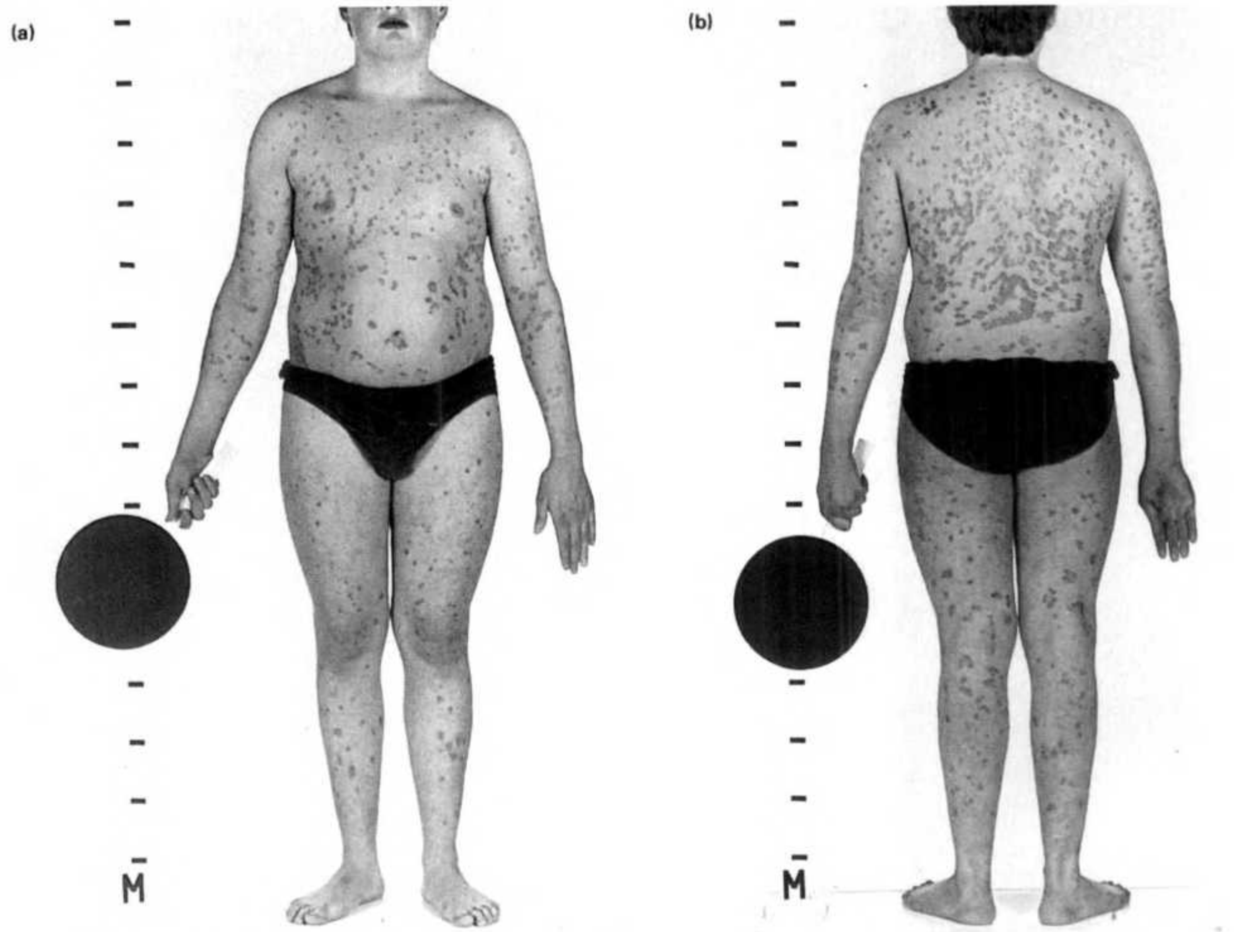

Figure 1. Patient 9: (a) front and (b) rear view. This patient, with small plaque psoriasis, was assessed by the four observers as having an average 19, 12, 34, and 41% body surface involvement with psoriasis. The plaque traced area was 5% and the photographic method estimated 8%.

Photographs

Black-and-white anterior and posterior whole body photographs measuring 21 × 30 cm were taken by a professional photographer in a studio using the same lighting and posture for each patient. The patient held a 22·5 cm-diameter calibration disc, with a handle attached (Fig. 1). Photographs were analysed for extent of psoriasis, excluding the head, neck and groin, using the image analysis system. A light pen was used by the operator to outline the body site to be assessed on the image analysis colour monitor. A colour monitor was used so that grey tone differences on the black and white photograph could be colour coded and more easily seen by the operator. The image was enhanced by the image analysis system which automatically joined any incomplete plaque outlines. The image analysis system was able to subtract the plaque outlines from the body outline so that this was not interpreted as a large plaque. Occasionally the image analysis system misinterpreted skin shadows as plaques or joined up adjacent plaques. The operator had to check to ensure that the computer derived image was identical to the original photograph and if necessary, adjustments were made using the light pen before area estimation. The light pen, or electronic pointer was used to draw and erase, on the displayed image, with a single-pixel accuracy in order to delineate the plaques correctly. There were 512 × 512 pixels, or picture elements, in the image when stored in the computer. By comparing the number of pixels in the outlined plaque area to the number in the known area of the circular calibration disc the computer was able to give an area of involved skin in m^2. As pixels in the Magiscan system were square there was no need to make a correction for orientation. Each of the six body sites were analysed separatedly, which took 50 min/patient, and the six sites added to give a measure of the whole body surface area involvement.

Image analysis system

The Magiscan image analysis system (Joyce-Loebl Ltd, Team Valley, Gateshead, U.K.) comprises a purpose-built image processing unit connected by a special parallel interface to a control processor. The image processing unit contains 2048 × 2048 pixels in its image memory, each pixel comprising up to 16 bits of data. Images are digitized with 8-bit resolutions (256 grey levels) from a monochrome TV camera, using the normal CCIR video standard (25 frames per s). The image processing unit displays images with colour overlays on a high resolution colour monitor. Thus, for example, detected plaques areas were shown in red over the monochrome image of the patient, while the operator edited the red overlay with the light pen. The control processor is a PC/AT-compatible computer running MS-DOS. It has 1 Mbyte of RAM, a 1·4 Mbyte floppy disc drive and a 40 Mbyte hard disc.

Conversion into percentage body surface area

Standing nude body height and weight were measured in each patient and the total body surface area estimated from these results using the Dubois formula.[4] This area was reduced by 10% to allow for the excluded area of the head, neck and groin, giving the corrected body surface area. The area of involved skin, measured in m^2, obtained from the photographic and tracing methods was then expressed as a percentage of this corrected body surface area.

Statistical methods

Day-to-day differences within observers' and between the four observers' estimations of surface area were analysed using two-way within-subject analysis of variance. The differences between the traced surface area and the observers' estimations were compared using a comparison of mean and differences.[5] Using this method, the mean value of the differences indicates the bias between the two methods and the spread of these differences is indicated by the position of the two lines of agreement, the position of which is calculated from the formula 1·96 × SD, between which 95% of the differences will lie, if the differences are normally distributed. If the difference between these lines of agreement is clinically acceptable then the two measurement methods being compared can be used interchangeably.

Results

Rule of nines

The 10 in-patients were assessed by four observers who estimated mean total percentage involvement with psoriasis as 20% (range 3–49%), 14% (3–28%), 23% (5–54%) and 33% (7–69%), respectively (Table 1). These differences were highly significant ($P<0{\cdot}001$ ANOVA). By contrast there was no significant difference between area estimations made on day 1 and day 2 with differences of just 1–2% ($P>0{\cdot}05$ ANOVA) (Table 1).

Plaque tracings

The plaque traced areas for the 10 in-patients ranged from 0·025 to 0·278 m^2. Expressed as a percentage of the body surface area, this gave a range of 1–21% (mean 9%) body surface area involved by psoriasis. The contribution varied with body site, so that on average in the 10 patients each arm contributed 0·59 and 0·73%, each leg 1·38 and 1·45% and the anterior and posterior trunk 2·4 and 2·6%, respectively.

Photographs

The total surface area involved by psoriasis ranged between 0·015–0·23 m^2 (mean 0·11 m^2), or 1–15% (mean 7%) for the 10 patients (Table 1). At the six different body sites the mean percentage body surface area involved by psoriasis in these 10 patients was: arms 0·43 and 0·76%, legs 0·94 and 0·92%, anterior trunk 1·92% and posterior trunk 2·25%.

Comparison between plaque tracings and rule of nines assessment

A total of 80 surface area assessments were made on the 10 patients by the four observers over the 2-day period. On every occasion all the observers overestimated the area involved compared to the plaque traced areas. This overestimate was greater than twice the plaque traced areas on 49/80 or 62% of the observations. To determine the degree of bias and spread of this overestimate the difference between the plaque tracings and observer values was plotted against their average for each observer (Fig.2). Observers 1, 3 and 4 overestimated by a mean of 11, 14 and 24%, respectively, and the range of

Table 1. Comparison of estimations of involved surface areas by clinical observers, plaque tracings and clinical photograph. All values are plaque surface area as a percentage of the corrected body surface area

	Observers								Plaque	
	1		2		3		4			
Patient	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Tracing	Photo
1	25	29	28	26	33	27	45	40	21	15
2	16	15	9	9	18	18	25	14	8	3
3	22	25	18	24	23	29	49	46	11	9
4	13	22	14	19	20	19	33	38	12	8
5	3	3	3	5	5	6	11	11	1	2
6	20	16	14	15	23	20	23	24	10	11
7	19	13	12	12	26	20	31	31	8	3
8	7	9	4	6	7	6	7	7	5	1
9	20	17	11	12	32	36	41	40	5	8
10	43	49	16	16	39	54	69	68	10	13
Mean	19	20	13	14	23	23	33	32	9	7

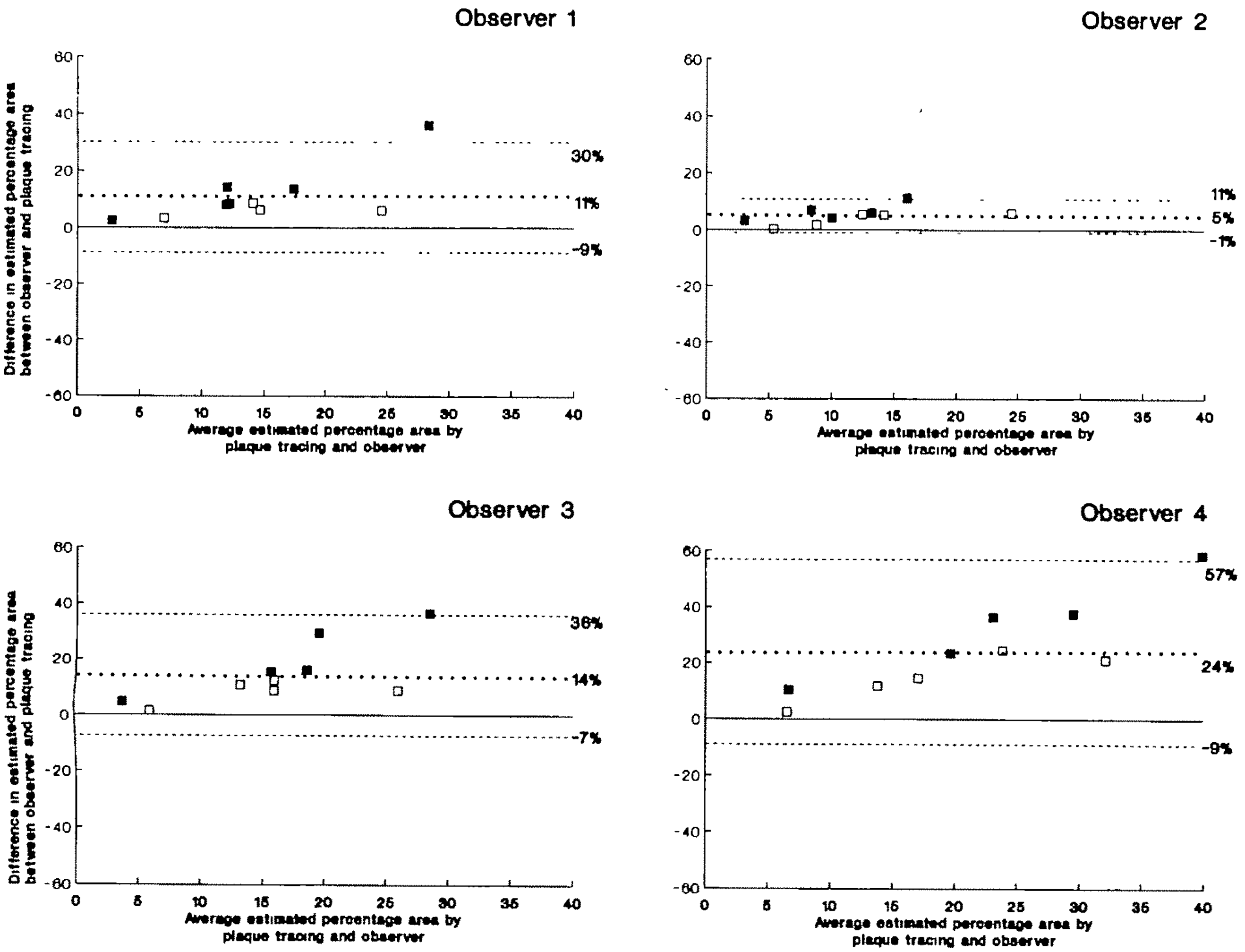

Figure 2. Comparison of the observer estimates of involved surface area and traced surface area. The difference between observer estimates and plaque tracings of involved surface area have been plotted against their average. The mean value of the differences (· · · · ·) indicates the bias between the two methods and the spread of these differences is indicated by the position of the hatched (-----) 1·96 × SD lines. Patients with small plaque (■) and large plaque psoriasis (□).

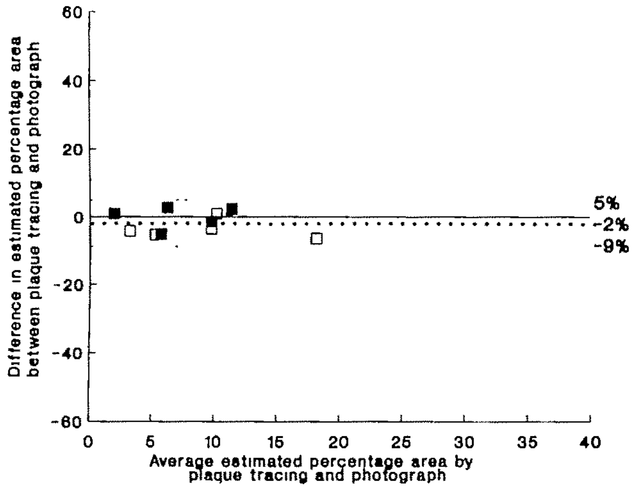

Figure 3. Comparison of image analysis estimate of involved surface area from whole body photographs and traced surface area. The difference between photographic estimates and plaque tracings of involved surface area have been plotted against their average. The mean value of the differences (···) indicates the bias between the two methods and the spread of these differences is indicated by the hatched (-----) 1·96×SD lines Patients with small plaque (■) and large plaque psoriasis (□).

the limits of agreement were 39, 43 and 66%, respectively. These differences between the observers' estimates and the area obtained from the plaque tracing would not be clinically acceptable and thus these two methods could not be used interchangeably. By contrast observer 2 overestimated with a bias of only 5% with limits of agreement between 11 and −1% (Fig. 2). All observers made their greater degree of error when assessing patients with small plaque psoriasis, when a 3·8-fold overestimate was made, compared to the 1·7-fold overestimate obtained in patients with large plaque psoriasis (Fig. 2). This is best illustrated by the patient illustrated in Figure 1 whose traced surface area was 5% compared to observer estimates of between 12–41% (Table 1).

Comparison between plaque tracings and clinical photographs

The mean involved surface area for the plaque tracings was 9% (1–21%) and for the clinical photographs it was 7% (1–15%) (Table 1). In nine out of 10 patients the clinical photograph areas were within 5% of the plaque traced areas. Comparison of the differences between these methods and their average value (Fig. 3) shows that the clinical photographic method underestimates the area affected by a mean of −2% with limits of agreement ranging from 5 to −9%.

Discussion

This study shows that compared to the value obtained from plaque tracings the assessment of the extent of psoriasis by untrained clinicians using the rule of nines is inaccurate. Computerized image analysis of whole body photographs gives results approaching those acheived by image analysis of traced plaque outlines. No method used in this study can be assumed to have precisely measured the involved body surface area, although the plaque tracing method is probably the most accurate.

The total involved traced surface area in our 10 patients, all of whom had been admitted for treatment, ranged from 1 to 21% which is much less than might be expected from previous studies of disease extent assessed using the rule of nines.[3] While disease extent is not always related to patient disability, because a small area of involvement on an exposed site may be more troublesome than extensive involvement of covered skin, the extent of psoriasis is probably the most easily measured index of patient handicap. Other methods of area assessment such as point counting[6] have been proposed and may provide a simple alternative that needs to be compared with image analysis. The rule of nines was developed for estimating the surface area of burns[7] and is an approximation of the previous direct measurement of body surface area.[2] The present study shows that, particularly in small plaque psoriasis, this method cannot be used by untrained observers, possibly because observers have to be able to group together by eye the multiple small, discrete and scattered plaques to assess the extent of psoriasis. Current methods of assessment of severity of psoriasis, including the PASI score, incorporate an assessment of involved surface area based on this rule.[1] In the present study all observers using the rule of nines overestimated the proportion of skin involved by psoriasis by up to 11-fold compared to the areas obtained from plaque tracings and there was up to a fourfold difference between observers. In three observers the differences between the plaque tracing and observer values were so great that the two methods could not be used interchangeably. By contrast observers' estimates showed little day-to-day variation although because there was only a 24-h interval between observations and patients were not studied in a group, recall bias cannot be excluded. One observer achieved an accuracy comparable to that obtained by the image analysis of clinical photographs suggesting that the rule of nines could be used if observers were first trained and their accuracy tested using this technique.

Plaque tracings had to be drawn, photographed, photocopied to enhance black and white contrast and examined carefully to ensure that the plaque outlines were continuous before the involved areas could be measured using the image analyser. It took 2 h per patient to complete these various stages, underlining the fact that this is not a method to be used routinely in large studies. Compared with the plaque tracing method the photographic method slightly underestimated the traced areas, as would be expected when comparing a two-dimensional with a three-dimensional image. However, these differences were much less than those seen using the rule of nines and in nine subjects these differences were less than 5%.

The Magiscan system is an industrial imaging system that was adapted for use in this study. It was more successful than previously described systems[3] but has two main disadvantages in that a large amount of operator interaction was needed and that high quality photographs, with clear plaque outlines and minimal body shadows were required. Manual editing of the computer-enhanced clinical photographs took 50 min per patient and without such editing small plaques would be merged and assessed as one large plaque. The image analysis system was not developed specifically for this type of work. It is likely that software could be developed for this or similar equipment to enable the rapid measurement of either the area in m^2 of a calibrating disc, and hence plaque surface area, or the percentage body surface area involved by comparing the plaque extent with the apparent total body surface area measured directly from the photograph. A different image analysis approach would make it possible to produce a system that does not depend on the absence of body shadows. By using a coloured image and comparing the ratio of green and red light, areas of similar coloured normal skin can be identified despite differences in light intensity. Colour differences due to psoriatic plaques will have a different green/red ratio and thus can be distinguished from the surrounding normal skin.

In conclusion, this study demonstrates that untrained observers cannot accurately estimate total involved skin surface area in patients with psoriasis, whereas image analysis of two-dimensional photographs shows an accuracy similar to that obtained from traced plaque outlines.

Acknowledgments

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References

1 Fredriksson T, Pettersson U. Severe psoriasis: oral therapy with a new retinoid. *Dermatologica* 1978; 157: 238–44.

2 Berkow SG. A method of estimating the extensiveness of lesions (burns and scalds) based on surface area proportions. *Arch Surg* 1924; 8: 138–48.

3 Marks R, Barton S, Shuttleworth D, Finlay AY. Assessment of disease progress in psoriasis. *Arch Dermatol* 1989; 125: 235–40.

4 DuBois D, DuBois E. Clinical calorimeter. A formula to estimate the approximate surface area if height and weight be known. *Arch Intern Med* 1915; 17: 863–71.

5 Bland J, Altman D Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; i: 307–10.

6 Bahmer F. The size of lesions or point counting as a step toward the solution of the PASI problem. *Arch Dermatol* 1989; 125: 1282–3.

7 Wallace AB. The exposure treatment of burns. *Lancet* 1951; i: 501–4.

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Computer-assisted image analysis of skin surface replicas

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Summary Using a rubber-based dental impression material, negative surface impressions were made of demarcated areas of skin and prepared as dry specimens for scanning-electron microscopy. Electron micrographs were taken at low magnification and, using a programed computer-assisted image analyser, it was possible to represent quantitatively the topography of that particular body site according to the degree of circularity of the various geometric subunits. This technique was most applicable to skin topography with a reliable geometrical pattern of triangles and squares, a feature of hair-bearing surfaces in general and in particular, the antecubital fossa. Using standardized trauma of various types the origin of skin surface markings were found to be located in the dermis. The technique was applied to the healing of experimental trauma produced by tape stripping, the resolution of clinical eczema and a comparison of steroid-treated and untreated tape-stripped skin.

The skin surface is characterized by specific regional patterns which can be broadly divided into hair-bearing, flexural, frictional and dermatoglyphic surfaces.[1] In addition a fifth category, the surface topography of the face, has been suggested.[2] The patterns present on the skin surface have been a subject of considerable interest for many years and a number of investigations have been carried out regarding the changes in these patterns in various diseases. Replication techniques have been widely used, but the majority of these studies have been qualitative.[1–9] Some attempts have been made to develop quantitative approaches using various engineering devices such as surfometers[10] and profilometers.[11] Qualitative studies have clearly shown that the basic skin patterns are disrupted by disease processes and return with treatment to apparent normality. The need to quantify these changes is obvious, particularly to evaluate the different treatments used. The various impression methods show the patterns as they appear on the skin surface which are lost and regained as the disease runs its course. However, surfometer and profilometer studies do not quantitate these patterns, and measure the variations at the surface of the skin. The advent of readily available computer-assisted image-analysis systems has prompted us to re-examine this problem and to attempt to assess quantitatively skin surface patterns. We have used a rubber-based fine detail dental impression material to produce permanent models of the skin surface. The virtue of this technique compared to biopsy studies is that sequential impressions can be obtained from the same skin site because the method is non-invasive.

Methods

Replication

Dental impression material, ESPE Impregnum F, was mixed according to the manufactuer's instructions and applied to a demarcated region of the skin. The negative impressions were left in 15% aqueous potassium hydroxide solution overnight to remove any adherent keratinocytes, dried and prepared as dry specimens for scanning-electron microscopy. Light microscopy and direct photography did not yield images of sufficiently high resolution for automated analysis and a wide range of other techniques were also studied.[3]

Scanning-electron microscopy

The specimens were fixed to stubs with double-sided Sellotape, surrounded by liquid DAG and sputter coated in an atmosphere of argon for three 1-minute intervals at a current of 40 mA using an Edwards S150 Sputter Coater. They were photographed at a magnification of × 20 using a Philips 501 scanning-electron microscope at zero degree tilt and a voltage of 15 kV.

Image analysis

A Seescan computer-assisted image analyser was used to

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assess automatically the electron micrographs. It was operated by a Task Programing Language (TPL), designed so that the operator preselects the desired mathematical function and a manual or automatic analysis of images within a certain grey scale range is performed. The triangular images distinctive to the hair-bearing surfaces were measured for their degree of circularity. The problem with this function is that the computer regards any small surface contaminant as a near perfect circle. To avoid this potential error, the area of the object being measured was combined with its degree of circularity. This mathematically separates those objects that have a high degree of circularity but very small areas, from the objects of interest that form the recognizable pattern. The expression combining these functions was termed Expression 2 which is mathematically equivalent to: EXP $2 = 4 \times \pi \times A \times A/P \times P$, where A = area and P = perimeter.

Nature of normal skin surface markings

The normal skin from a healthy volunteer was subjected to various traumas: tape stripping, the application of liquid nitrogen and laser burns, and these were compared with sites at which a basal cell carcinoma had been treated with X-irradiation and with an old tattoo site. The progress of these experimental lesions was followed by making Impregnum impressions at various intervals for a period of several weeks. Normal skin biopsies were obtained from cadavers following Impregnum impressions. These were sectioned horizontally and stained with CEA antigen to demonstrate sweat gland ducts. The slides were compared with the Impregnum impressions.

Quantitation of normal skin markings

Impregnum impressions were made from a demarcated area of the antecubital fossa and the dorsum of the hand daily for 5 days in two normal volunteers with no history of skin disease at these sites. The impressions were examined under the scanning-electron microscope and the same area was photographed and used for subsequent image analysis.

Healing eczema and tape-stripped skin

Skin surface impressions using ESPE Impregnum F were made daily from the tape-stripped skin on the dorsum of the hand of a patient with atopic eczema. Similar daily impressions were made before and afterwards from an area of tape-stripped skin in the antecubital fossa of a similarly aged control. The tape stripping was carried out with 1-cm pieces of Sellotape applied to the same site and vigorously removed. This was repeated 20 times each with a fresh piece of tape.

A subsequent study compared the tape-stripped skin of both antecubital fossae, one side being treated daily with 0·5% hydrocortisone cream. The impressions were compared in the same way as those in the previous experiments.

Photographs for the studies on reproducibility were always taken from the same area using orientation markers to avoid observer bias and the tendency to select 'best areas'.

It was considered important when taking skin impressions to attempt to standardize the position of the skin because tensions pulling in different directions could distort the surface patterns. The subjects were kept in relaxed positions with no tension and no muscle contraction. Comparable areas of skin were always examined in similar postures.

Ethical committee approval was obtained for all these studies and informed consent was given by all the patients.

Results

Nature of normal skin surface markings

It was observed that the regular skin surface markings seen on the palms and soles represented ridges on which sweat ducts opened. Horizontal sections showed that the sweat glands were arranged along the rete ridges. This pattern was complicated on hair-bearing surfaces by the presence of the hair follicles, which broke up the regular pattern of the rete ridges into rete pegs with the original skin surface markings radiating out from these and bearing the sweat glands.

All modalities of trauma disrupted skin surface markings, but only those which significantly damaged the dermis caused loss of the markings that persisted for up to 5 months.

Quantitation of normal skin markings

The surface markings of the antecubital fossa and the dorsum of the hand did not appear to alter from day to day in both subjects. When the means and ranges for Expression 2 were plotted as a value for each intraindividual site and as a summation of successive fields of view for that site, it could be seen that there was more inherent variation in the surface markings on the back of

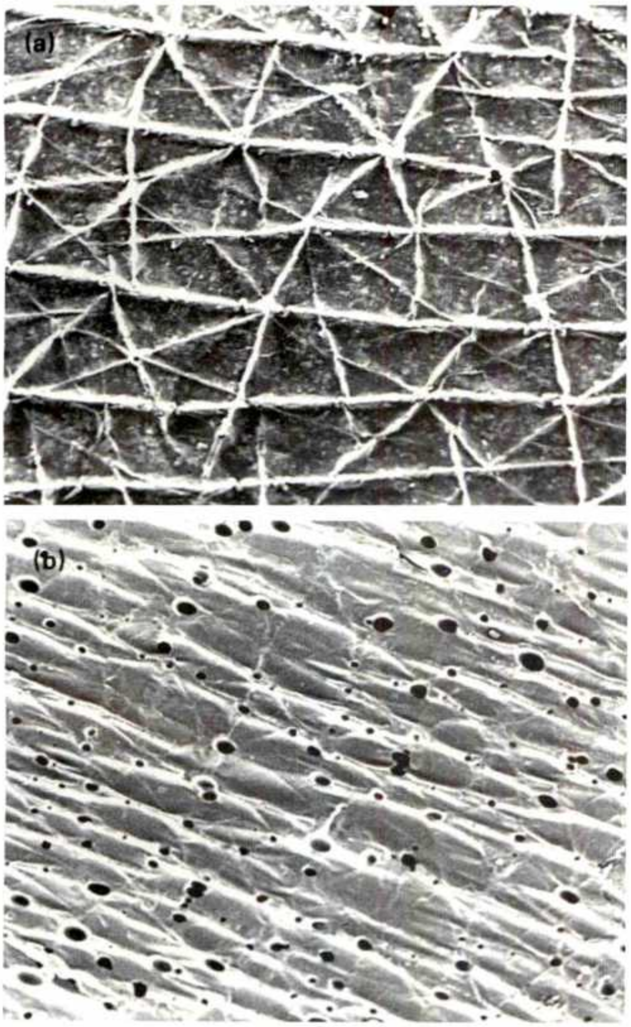

Figure 1. (a) SEM (×20) from the dorsum of the hand of a normal individual. (b) SEM (×20) of an eczematous lesion on the dorsum of the hand before treatment.

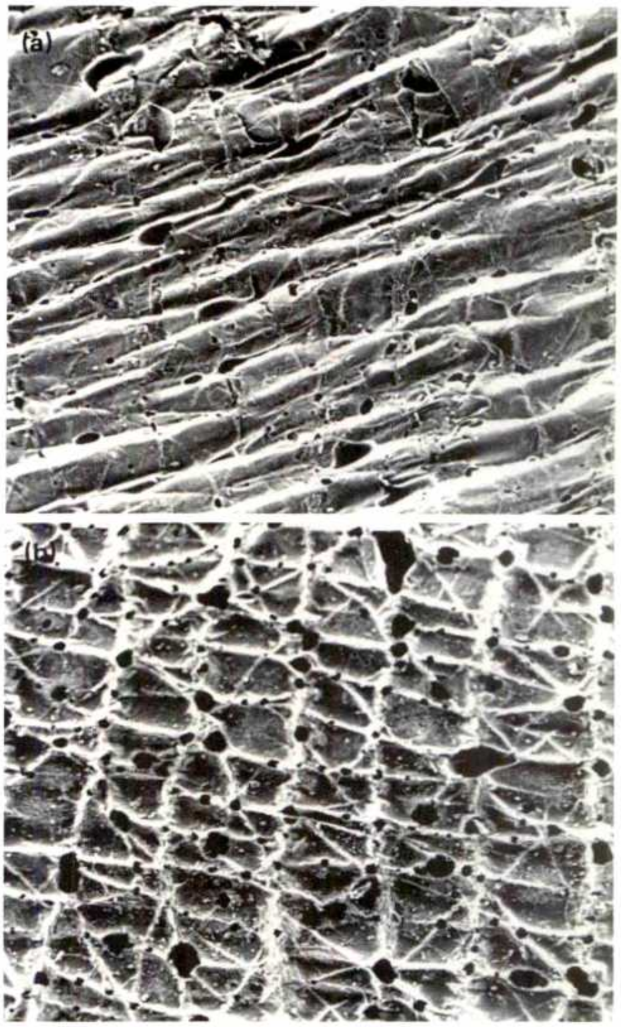

Figure 2. (a) SEM (×20) of an eczematous lesion on the dorsum of the hand after 3 days treatment (same site as Fig. 1(b)). (b) SEM (×20) of an eczematous lesion on the dorsum of the hand after 5 days treatment (same site as Fig. 1(b)).

the hand than the antecubital fossa. The individual variation from area to area within a given topographical site, although small, became insignificant when three fields of view of the antecubital fossa were averaged, representing an area of 3 cm^2. There was greater variation in the topography of the dorsum of the hand and this became significant when five fields representing an area of 5 cm^2 were averaged. The variation between sites in the antecubital fossa was so small that a single impression representing an area of 1 cm^2 could be used with confidence.

Healing eczema and tape-stripped skin

The eczematous process appeared to disrupt the small unit structures on the hair-bearing surface of the dorsum of the hand. The triangles and squares were 'opened up' and more elliptical figures with ill-defined boundaries were evident. However, on healing the characteristic triangular unit structures of a hair-bearing surface reformed (Figs 1 and 2). Quantitation showed that the middle ranging values that represent the triangular unit structures were lost in frankly eczematous skin and reappeared as the skin healed.

Tape stripping produced an erythematous tender area of skin. During the following 5 days the skin surface became progressively more rough and this was mirrored by a gradual breakdown of the skin patterning. The small unit structures were lost and replaced by a large coarse patterning of ill-defined units (Fig. 3). As the skin

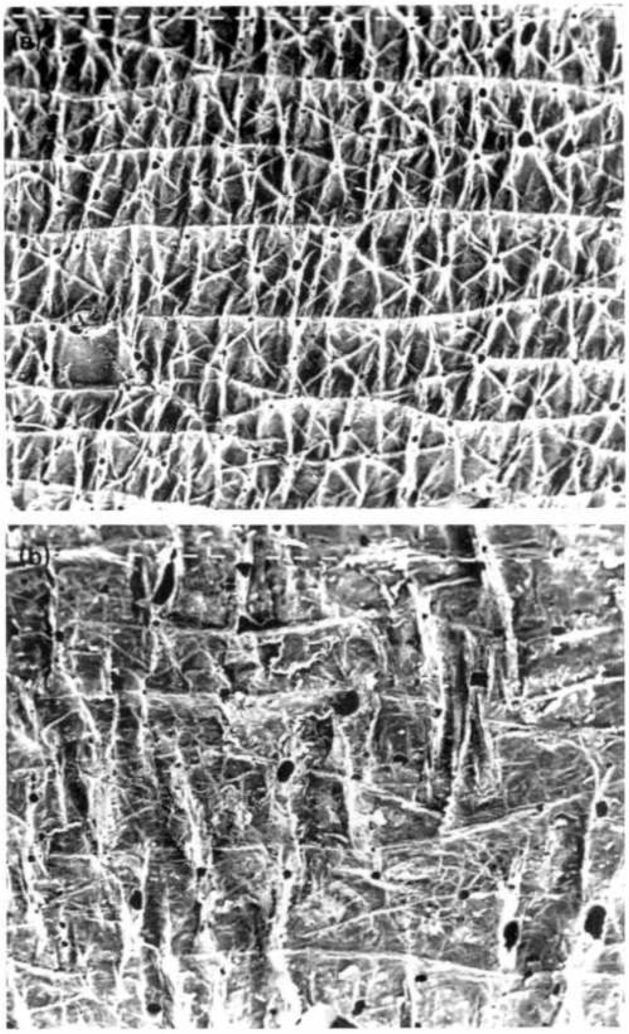

Figure 3. (a) SEM (× 20) of the antecubital fossa of a normal individual. (b) SEM (× 20) of the antecubital fossa of a normal individual 5 days following tape stripping (see text). (Same site as Fig. 3a.)

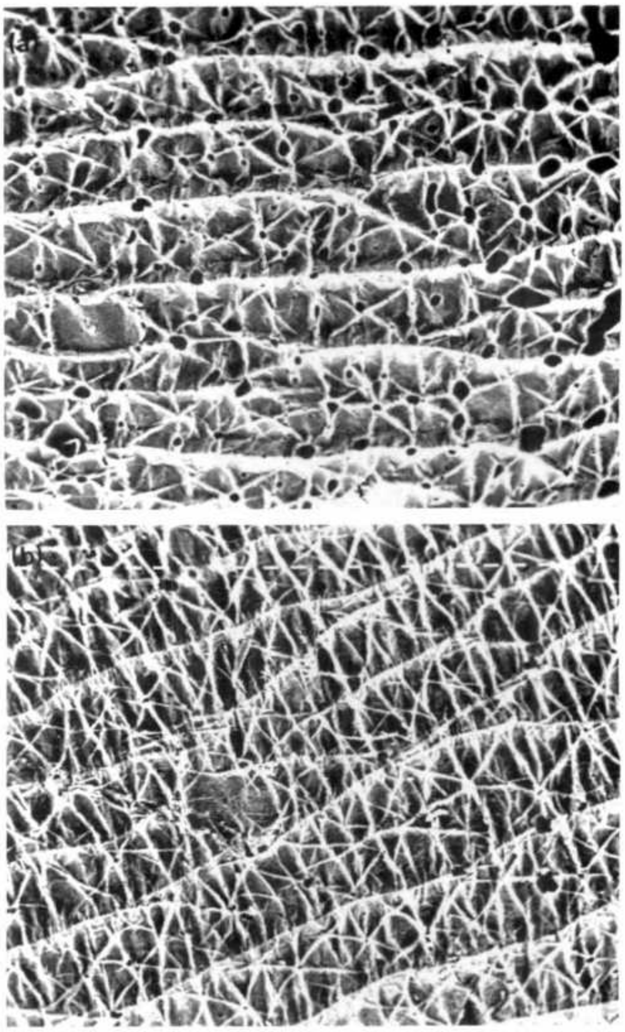

Figure 4. (a) SEM (× 20) of the antecubital fossa of a normal individual 10 days following tape stripping (see text). (Same site as Fig. 3.) (b) SEM (× 20) of the antecubital fossa of a normal individual 14 days following tape stripping (see text). (Same site as Fig. 3.)

became clinically normal, by day 15 post-stripping, the surface markings regained the normal geometry of small unit structures for that site (Fig. 4).

The application of topical steroid to tape-stripped skin resulted in a return to the normal pattern 3 days sooner than the non-treated control area.

Discussion

Skin surface markings have been extensively studied but there have been limitations as to their analysis. With the development of the Seescan image analyser with its Task Programing Language (TPL) these limitations have been largely overcome and it is possible to analyse quantitatively very complex patterns. The reproducibility of the techniques used in our studies was good and the findings on the topography of the skin in the antecubital fossa agree with skin surface studies.[2] No attempt was made to quantitate other regional patterns that are more variable, although there seems no reason to suppose that this could not be overcome by taking larger or more numerous samples. The decision to use circularity as a form factor for quantitation was used for convenience. The pattern on the dorsum of the hand diminishes with age due to various factors, particularly photoageing. The lines become less pronounced and the geometrical pattern becomes less exact. This, in conjunction with the greater inherent pattern variability at this site, suggests that the back of the hand is a less suitable area for the application of the technique compared to the antecubital fossa. Our findings regarding the origin of skin surface markings are in accordance with common experience in that it is well known that scars, which disrupt surface markings, only occur with dermal damage. In actinic damage with solar elastosis, the epidermis is thin and skin surface patterns are lost.

The automatic image analysis of biological structures has considerable limitations. A low-power electron micrograph was chosen for analysis because light micrographs at the same magnification gave a greater degree of variability. Minor variations in the skin surface and the nature of the replication material were probably 'smoothed out' by the technique of preparation for scanning-electron microscopy. Obviously electron microscopy is an expensive and complex technique compared to simple photography, but for establishing the technique and reducing 'noise' levels, it is superior. However, it is perfectly possible to perform image analysis using impressions or photographs if larger sample areas are used.

The image analyser recognizes and measures images according to their shade of grey which is given a value represented on a grey scale. This set of values ranges from 0 to 127 where 0 is white and 127 is black with the shades of grey lying in between. Objects are measured only if they lie within a previously selected grey-shade range and so it is necessary for the operator to set the range to include the objects of interest. Inevitably, several objects share a common grey shade and some objects which are not of interest are measured. This can be avoided by making manual measurements but this increases the subjectivity of the technique. To minimize this effect the technique was made more selective by combining the functions of area and circularity which mathematically picked out the images of interest. Objects that are 'contaminants' are also measured and the technique is more reliable when used to show trends such as healing lesions, rather than used in isolation. Using the system in this way it was possible to follow the changes as eczema or tape-stripped skin healed and to show some effect of topical steroids on this process.

Image analysis of the topographical changes in eczema and tape-stripped skin was sensitive enough to show a numerical difference between grossly lesional and clinically normal skin. This was represented by an absence or presence, respectively, of middle-range values for Expression 2 (circularity × area). However, image analysis of the more subtle changes from one day to the next when the condition was almost healed did not reveal any regular differences: these were only apparent at intervals of 3–4 days.

Quantitation of the surface microtopography of eczematous and tape-stripped skin showed a loss of the middle range values representing the triangular unit structures and this gradually returned as the skin became clinically normal. It is important to note that clinical restoration of tape-stripped skin after 14 days does not equate with restoration of the water barrier to full function.[12] The virtue of the technique rests upon demonstrating a quantitative rather than a qualitative set of changes.

Acknowledgments

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References

1 Wagner G, Goltz RW. Human cutaneous topography: a new photographic technique. Observations on normal skin. *Cutis* 1979; **23**: 830–43.

2 Chinn HD, Dobson RL. The topographic anatomy of human skin. *Arch Dermatol* 1964; **89**: 267–73.

3 Murphy R. Skin surface markings: a qualitative and quantitative approach. *BSc dissertation*, University of Sheffield, 1989.

4 Facq JM. New pressure-less replica technique for the study of human skin. *The Toilet Goods Association* 1967; 47: 9–16.

5 Marks R, Dawber RPR. Skin surface biopsy: an improved technique for the examination of the horny layer. *Br J Dermatol* 1971; **84**: 117–23.

6 Nayler JR. Applications of the skin surface replica technique in dermatology. *J Audiovisual Media in Medicine* 1984; 7: 21–6.

7 Tring FC, Murgatroyd LB. Psoriasis: change in surface microtopography. *Arch Dermatol* 1975; **111**: 476–80.

8 Tring FC, Murgatroyd LB. Surface microtopography of normal human skin. *Arch Dermatol* 1974; **109**: 223–6.

9 Oberste-Lehn H. Dermoepidermal interface. *Arch Dermatol* 1962; **92**: 651–7.

10 Mark R, Pearse AD. Surfometry. A method of evaluating the internal structure of the stratum corneum. *Br J Dermatol* 1975; 92: 651–7.

11 Prall JK. Instrumental evaluation of the effects of cosmetic products on skin surfaces with particular reference to smoothness. *J Soc Cosmetic Chemists* 1973; **24**: 693–707.

12 Grice K, Sattar H, Sharratt M, Baker H. Skin temperature and trans-epidermal water loss. *J Invest Dermatol* 1971; 57: 108–10.

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Red tattoo reactions: X-ray microanalysis and patch-test studies

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Summary Eighteen patients who developed cutaneous reactions to red tattoos were studied to identify the chemicals responsible for the reactions to modern red tattoo pigments. Biopsies from the tattoos were examined histologically and the chemical composition of the red pigments was analysed by X-ray microanalysis. A variety of metallic elements including aluminium, iron, calcium, titanium, silicon, mercury and cadmium were detected. Patch tests were performed to the relevant chemicals in nine cases, and only one patient reacted to mercury. This study demonstrates that although reactions to mercury still occur, other red dyes containing a variety of inorganic pigments may provoke a cutanous inflammatory response.

Tattooing has been practised for over 8000 years and remains a common practice in various countries and cultures.[1] The introduction of foreign substances into the skin can promote a toxic or an immunological response and reactions to various tattoo pigments, including red mercuric sulphide,[2] green chromium salts,[3] yellow cadmium sulphide,[4] blue cobalt salts,[5] purple manganese salts[6] and alluminium-containing pigments[7] have been described.

Various histological types of tattoo reaction are recognized. In general, dermal changes predominate, but epidermal changes such as acanthosis or spongiosis can also be seen.[8] The chronic inflammatory cell infiltrate can be nodular[9] with a well defined Grenz zone, lichenoid[10] or granulomatous.[11] Occasionally, tattoo reactions have the same histological and immunofluorescence appearances as idiopathic lichen planus.[10,12] Granulomatous tattoo reactions take two forms. A foreign-body reaction to pigment can produce numerous giant cells containing large quantities of pigment.[13] An apparent hypersensitivity granulomatous reaction can also occur, characterized by dense aggregates of epithelioid cells, a thin peripheral ring of lymphocytes and few giant cells.[3,13,14] These appearances may be indistinguishable from the tattoo involvement seen as a Köbner response in sarcoidosis[15,16] and further investigations may be necessary to exclude systemic disease.

Occasionally, the dermal infiltrate may be so dense and nodular as to suggest a diagnosis of cutaneous lymphoma. Usually, there are sufficient clinical and histological criteria to diagnose pseudolymphoma although immunocytochemistry may be necessary to confirm the diagnosis.[17,18]

In a 2-year period up to December 1988, 18 patients with inflammatory reactions confined to red tattoos were referred to the Stoke Dermatology Unit. Biopsies were taken from all of the tattoo reactions and examined histologically. X-ray microanalysis of biopsy material was also performed in an attempt to define the nature of the foreign materials within the skin. The theoretical aspects of this procedure have been described previously[19] and the technique has been used to study tattoos.[7,9]

Methods

Patients

All patients were referred by their family practitioners and presented with similar symptoms and signs. Patients complained of discomfort and swelling affecting their red tattoos. Examination revealed swelling, induration and tenderness of the red areas of the tattoos; other colours were not involved. One patient (Case 18) subsequently developed systemic sarcoidosis but all others remained well throughout the study.

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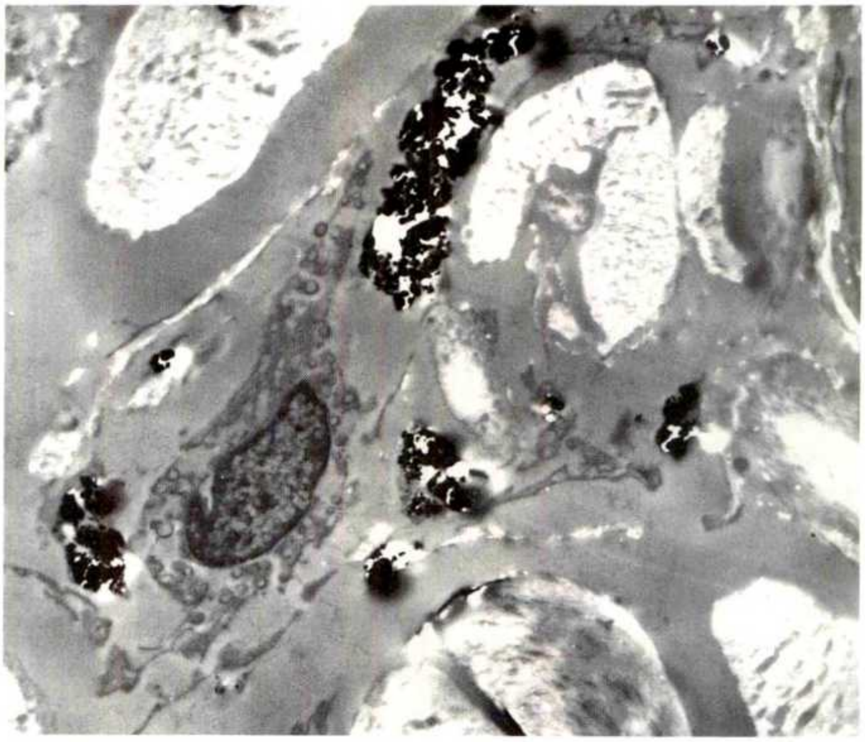

Figure 1. Electron micrograph showing large amounts of tattoo pigment, mostly within a dermal macrophage (× 9600).

Histology

Biopsies were taken from inflamed portions of the red tattoos under local anaesthesia. Specimens were fixed in buffered formalin–saline and processed to paraffin wax using standard techniques. Sections were stained with haematoxylin and eosin. In the cases where immunofluorescence studies were performed, portions of the biopsies were sent unfixed for analysis and examined using standard immunofluorescence techniques.

X-ray microanalysis

Histological sections taken from the formalin-fixed, paraffin-processed biopsies of tattoos were examined microscopically to select areas containing pigment. These sections were processed to EMIX epoxy resin through xylene and then propylene oxide. Capsules filled with resin were inverted over the relevant area of the section and polymerized overnight. The blocks with adherent section were removed from the glass slide using a razor blade. Ultrathin sections of the relevant area were cut using a Reichert Ultracut and mounted on both copper and nickel grids to overcome elemental masking on analysis. Sections were examined ultrastructurally on a Phillips 400 transmission-electron microscope (TEM), and analysed using an Energy Dispersive Analysis of X-rays (EDAX) attachment (model 711) interfaced to an EDAX Peak Identification Computer (609A). Conventional electron micrographs were taken of relevant areas using plate film incorporated into the TEM (Fig. 1) and polaroid photographs were taken of the VDU showing the elemental peaks from EDAX (Fig. 2). Using this analyser only elements with atomic numbers between 11 and 92 can be detected.

Patch tests

Following the X-ray microanalysis, patch tests to the relevant chemicals and red tattoo dyes were performed in some of the cases. One of the local tattoo studios provided the red dyes allegedly used in six of the patients, although no formal records of the pigments used had been kept.

The following patch tests were applied using standardized techniques with readings at 48 and 72 h: mercury, 1% ammoniated mercury (mercuric chloride) in petrolatum; cadmium, 1% cadmium chloride in water; iron, 1 and 2% ferric chloride in water; aluminium, 10% aluminum sulphate in water; phosphorus, 1% phosphorus sesquisulphide in petrolatum; and tattoo pigments, 10% red pigments in water.

Results

The patient details, histology, X-ray microanalysis and patch-test results where appropriate are shown in Table 1.

Clinical features

Over a 2-year period, 17 males and one female patient were seen with inflammatory reactions confined to red tattoos. No reactions to other colours were seen during this period. Three patients had developed inflammation 12 years after the tattoos were performed; mercury was identified in two of these three tattoos. By contrast, most of the tattoos had been performed within 2 years of presentation, and only two out of these 15 contained mercury.

Photoaggravation of tattoo reactions

Seven patients noticed that their red tattoos became

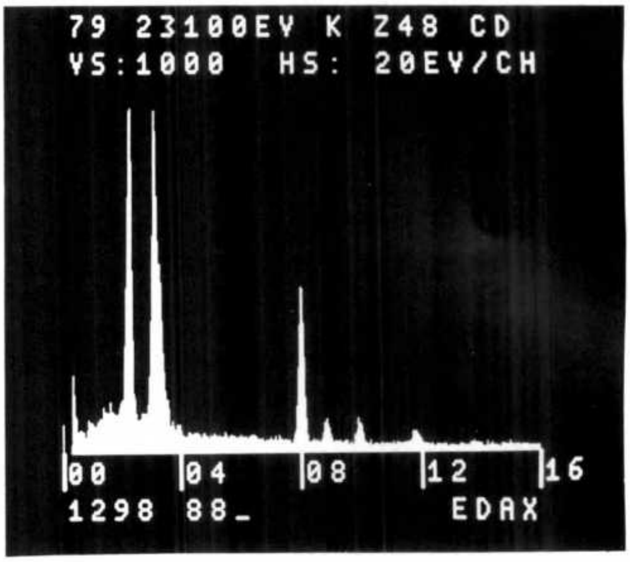

Figure 2. Photograph of the VDU showing the elemental peaks from the EDAX for Patient 9. The peaks (from left to right) correspond to mercury, cadmium and copper (present on the grid).

Table 1. Details of patients presenting with red tattoo reactions over a 2-year period to December 1988

Case	Age/sex	Duration of tattoo prior to reaction reaction months (m) years (y)	Photoaggravation	Histological pattern	X-ray microanalysis	Patch tests
1	29 M	12 m	Yes	Lichenoid	Fe/S/Ti	—
2	19 M	17 m	—	Lichenoid	Fe/S/Ti	—
3	19 M	12 m	Yes	Lichenoid	P	P Neg Tat.Pig.Neg
4	19 M	12 m	—	Lichenoid	Al/Si/Fe/Ca	—
5	22 M	12 m	—	Granulomatous	Ti	—
6	27 M	12 m	Yes	Granulomatous	Al/Si/Fe/Ca	—
7	26 M	14 m	—	Lichenoid	P/Al/Ca	Tat.Pig.Neg
8	22 F	24 m	No	Lichenoid	Al/P/S	P Neg. Tat.Pig.Neg
9	20 M	24 m	Yes	Lichenoid	Hg/Cd	Hg/Cd Neg Tat.Pig.Neg
10	26 M	6 m	—	Nodular	Al/P/S/Ca	Al/P Neg
11	33 M	15 y	—	Nodular	Hg/Cd	Hg Pos Cd Neg
12	20 M	12 m	No	Lichenoid	Al/Si/Fe/Ca P/S	—
13	20 M	18 m	Yes	Lichenoid	Al/S/P/Ti	Tat.Pig.Neg
14	18 M	12 m	No	Lichenoid	Al/Si/Fe/Ca	—
15	34 M	12 y	Yes	Lichenoid	Hg	Hg/Cd Neg Tat.Pig.Neg
16	22 M	18m	—	Lichenoid	Ti/Al/Si/Fe/Ca	—
17	33 M	24 m	Yes	Lichenoid	Hg/Cd	Hg/Cd Neg
18	32 M	15 y	No	Sarcoidosis	Fe	—

—, Not available; Tat.Pig, tattoo pigment; Pos, positive; Neg, negative; Fe, iron; S, sulphur; Ti, titanium; P, phosphorus; Al, aluminium; Si, silicon; Ca, calcium; Hg, mercury; Cd, cadmium.

more inflamed following sun exposure, and three of these attributed the onset of their tattoo reactions to sunlight.

Histology

The most common histological pattern was a lichenoid reaction, seen in 13 of the 18 patients. Immunofluorescence studies were performed in four of these cases and dermal IgA and IgM coated colloid bodies were seen in two biopsies. Three biopsies showed a granulomatous infiltrate, and one of these patients developed systemic sarcoidosis. The remaining two cases showed a nodular dermal lymphohistiocytic infiltrate with a Grenz zone.

X-ray microanalysis

Aluminium was found in nine biopsies, iron in eight, calcium in seven, sulphur and phosphorus in six, titanium and silicon in five, mercury in four and cadmium in three biopsies (Table 1).

Patch tests

Patch tests were performed in a total of nine patients. Seven patients had patch tests to elements detected in their red tattoos by X-ray microanalysis. One of these reacted to 1% ammoniated mercury. Six patients were patch tested to the red tattoo pigments allegedly used in their tattoos and none of these was positive.

Discussion

Reactions to mercury sulphide in the red tattoo pigments artificial cinnabar, vermilion or Chinese red have been well documented.[2,8] In the period 1960–68, out of 57 patients with reactions to red tattoos, 45 were sensitive to mercury on patch testing.[20] Because of the problems with mercury, alternative red dyes have been used for tattooing, including cadmium red (cadmium selenide), sienna/red ochre (ferric hydrate) and organic substances such as sandalwood and brazilwood. Despite the availability of these mercury-free alternatives, reactions to red tattoos continue to be reported.[9,10] We have performed the first large study of red tattoo reactions using histological techniques, X-ray microanalysis and patch testing.

One of the alternatives to mercury compounds is cadmium, which can be found in both yellow (cadmium sulphide) and red (cadmium selenide) tattoo pigments. Historically, cadmium has been identified in photosensitive reactions to tattoo pigments.[4,14] In our series, seven patients noticed that their red tattoos became more inflamed with sun exposure, and three of these attributed the onset of their tattoo reactions to sunlight. X-ray microanalysis studies on tattoo biopsies revealed cadmium and mercury in two patients, mercury alone in the third and a variety of elements in the remaining four biopsies. This suggests that photoaggravation of tattoo reactions is not confined to cadmium-containing tattoos. Further investigations would be necessary to distinguish photoallergic from phototoxic reactions.

Biopsies taken from the red tattoo reactions revealed a variety of histological patterns. In Case 18, the dermal changes were suggestive of sarcoidosis, with densely packed non-caseating epithelioid–cell granulomata associated with a sparse lymphocytic infiltrate. Further investigations revealed systemic sarcoidosis with pulmonary and joint involvement. This patient has been reported elsewhere.[21] In two patients, the granulomatous infiltrate showed features of a foreign-body reaction, with numerous giant cells containing large quantities of pigment. In the remaining 15 patients the dermal infiltrate was predominantly lymphohistiocytic. In two cases, this infiltrate was nodular with a Grenz zone and in the remaining 13 biopsies the infiltrate was lichenoid. In two patients, IgA- and IgM-coated colloid bodies were identified in the upper dermis.

There have been eight previously reported cases of lichenoid reactions to red tattoos.[10,12,22] In one case analysis of the tattoo pigment revealed mercury and patch tests to mercuric compounds were positive.[12] Although another case reacted to mercuric chloride patch tests, mercury was not identified in either the tattoo pigment or the biopsy material.[10] In our series, X-ray microanalysis demonstrated mercury in only three of the 13 lichenoid reactions, suggesting that this inflammatory response occurs as a reaction to a variety of red tattoo pigments.

In our series, seven of the patients had patch tests to elements identified in their tattoos by X-ray microanalysis. One patient with mercury and cadmium in his tattoos reacted to 1% mercuric chloride but not to 1% cadmium chloride. None of the other patch tests were positive. Six patients showed negative patch-test reactions to the red pigments allegedly used in their tattoos. However, because the tattooist had not kept formal records of the tattoo pigments used, the validity of these data can be questioned. Overall, these results support the argument that patch tests are not a useful investigation in patients with tattoo reactions.[10,12] In contact dermatitis, antigen-presenting cells within the epidermis initiate the inflammatory response and epicutaneous testing is justified. In tattoo reactions, the inflammatory response is triggered by material within the dermis and intracutaneous testing seems more appropriate.[7] As our patients' tattoo reactions responded to either topical therapy or surgery, we did not feel that intracutaneous testing was justified.

This study demonstrates that a variety of metallic compounds are found in red tattoo pigments (Table 1). The two tattoos performed in the 1970s contained mercury, but since then there has been a move away from using mercury-containing dyes. Despite this, two of the tattoos performed 2 years prior to presentation contained mercury. Ferric hydrate (sienna or red ochre) and cadmium selenide (cadmium red) are recognized mercury-free alternatives; iron was identified in the tattoos of eight patients and cadmium in three others. Of the other metallic elements detected by X-ray microanalysis, titanium, aluminium, silicon and calcium are all used in white tattoo pigments[10] and it is likely that these were added to red dyes to produce a lighter colour. A total of eight biopsies contained either phosphorus or sulphur, and in four cases the two were found in combination. This raises the possibility that phosphorus sequisulphide is used in some red tattoo pigments.

The X-ray microanalysis system used in this study does not detect elements with atomic numbers less than 11 and so cannot identify either carbon or organic dyes. With Patients 6 and 14, although electron microscopy detected large quantities of pigment within the dermis, the X-ray microanalysis only found traces of aluminium, silicon, iron, titanium and calcium. It is likely that in many of the patients, organic dyes were used in conjunction with red iron-containing compounds and white pigments containing aluminium, silicon and calcium. Organic dyes that have been used as alternatives to metallic compounds include sandalwood and brazilwood. Unfortunately, we have been unable to discover the origin or nature of the organic dyes used in our patients.

This study revealed 17 patients with reactions to red tattoos and no reactions to other colours. Despite the use of mercury-free alternatives, reactions to red tattoo pigments containing a variety of metallic elements and organic dyes continue to be a problem. The predominant histological picture in this series was the lichenoid tattoo reaction. As with previous studies[10,12,22] we were unable to link this reaction to any single metallic element or tattoo dye.

References

1 Levy J, Sewell M, Goldstein N. A short history of tattooing. *J Dermatol Surg Oncol* 1979; 5: 851–6.

2 Verdich J. Granulomatous reaction in a red tattoo *Acta Derm Venereol (Stockh)* 1981; 61: 176–7.

3 Loewenthal LJA. Reactions in green tattoos. *Arch Dermatol* 1960; 82: 237–43.

4 Bjornberg A. Reactions to light yellow tattoos from cadmium sulphide. *Arch Dermatol* 1963, 88: 267–71.

5 Rorsman H, Dahlquist I, Jacobsson S *et al* Tattoo granuloma and uveitis. *Lancet* 1969; ii: 27–8.

6 Schwartz RA, Mathias CG, Miller CH *et al.* Granulomatous reaction to purple tattoo pigment. *Contact Dermatitis.* 1987; 16: 198–202.

7 McFadden N, Lyberg T, Hensten-Pettersen A. Aluminium-induced granulomas in a tattoo. *J Am Acad Dermatol* 1989, 20: 903–8.

8 McGrouther DA, Downie PA. Reactions to red tattoos. *Br J Plast Surg* 1977; 30: 84–5

9 Slater DN, Durrant TE. Tattoos: light and transmission electron microscopy studies with X-ray microanalysis. *Clin Exp Dermatol* 1984; 9: 167–73.

10 Taafe A, Knight AG, Marks R. Lichenoid tattoo hypersensitivity. *Br Med J* 1978; 1: 616–18.

11 Goldstein AP Histological reactions in tattoos. *J Dermatol Surg Oncol* 1979; 5: 896–900.

12 Clarke J, Black M. Lichenoid tattoo reactions. *Br J Dermatol* 1979, 100: 451–4.

13 Madden JF. Reactions in tattoos. *Arch Dermatol* 1939; 40: 256–62.

14 Goldstein N. Mercury-cadmium sensitivity in tattoos: a photoallergic reaction in red pigment. *Ann Int Med* 1967; 67: 984–9.

15 Iveson JMI, Cotterill JA, Wright V. Sarcoidosis presenting with multiple tattoo granulomata. *Postgrad Med J* 1975; 51: 670–2.

16 Farzan S. Sarcoidal reaction in tattoos. *N Y State J Med* 1977; 77: 1477–9.

17 Blumental G, Okun MR, Ponitch JA. Pseudolymphomatous reaction to tattoos *J Am Acad Dermatol* 1982; 6: 485–8.

18 Rijlaarsdam JU, Bruynzeel DP, Vos W *et al.* Immunohistochemical studies of lymphadenosis benigna cutis occurring in a tattoo. *Am J Dermatopath* 1988; 10: 518–23.

19 Bleehen SS, Gould DJ, Harrington CL *et al.* Occupational argyria; light and electron microscopic studies and X-ray microanalysis. *Br J Dermatol* 1981; 104: 19–26.

20 Scutt RM. The medical hazards of tattooing. *Br J Hosp Med* 1972, 8: 194–202.

21 Sowden JM, Cartwright PH, Slater DN *et al.* Sarcoidosis presenting with a granulomatous reaction confined to red tattoos. *Clin Exp Dermatol* (in press).

22 Winkelmann RK, Harris RB. Lichenoid delayed hypersensitivity reactions in tattoos. *J Cutan Pathol* 1979; 6: 59–65.

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A double-blind, placebo-controlled trial of topical PUVA in persistent palmoplantar pustulosis

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Summary The effect of topical PUVA was investigated in the treatment of patients with persistent palmoplantar pustulosis (PPP). In this double-blind, placebo-controlled trial of 27 patients with PPP there was an overall improvement of the hands and feet in both the active and placebo-treated areas and little difference between them.

Persistent palmoplantar pustulosis (PPP) is a chronic dermatosis characterized by relapses and remissions and which presents to the dermatologist a considerable therapeutic problem. Oral psoralen photochemotherapy (PUVA) has been shown to be of value in the treatment of PPP in a placebo-controlled study.[1] However, oral administration of psoralens can produce a number of adverse reactions, many of which can be avoided by the topical use of the drugs. Several uncontrolled studies[1–4] have suggested that topical PUVA may be of value in the treatment of PPP. However, one uncontrolled study found topical PUVA to be ineffective.[5]

The aim of this study was to investigate how effective topical PUVA was in the treatment of PPP.

Methods

The study comprised 27 patients with bilateral symmetrical and therapy-resistant PPP for at least 1 year's duration of either the hands or feet or both. No other treatment was administered in the month prior to the start of the study and any topical treatment used during the course of the trial was applied bilaterally. Of the patients, 13 resumed having topical therapy at the start of the trial, nine with emollients and four with steroid preparations. All the patients gave informed consent.

Twenty-six patients received treatment to their feet and 18 to their hands. The extent and severity of the disease was assessed prior to treatment and after 4 and 8 weeks of therapy. The method by which percentage involvement was assessed is shown in Figure 1. For the feet, 2% was allocated to each toe and the sole was divided into nine equal parts each representing 10%. For the hands, the fingers, thumb and thenar eminence were each allocated 10% and the palm was divided into eight equal parts representing 5% each. The total active and resolving pustule counts were recorded and a grade calculated for PPP according to an erythema, scaling and fissuring score, where 0 = normal or near normal skin with minimal scaling, 1 = erythema with scaling only, 2 = erythema, scaling and shallow fissures and 3 = erythema, scaling and deep fissures. Subjective symptoms were assessed by the patients using a 10 cm horizontal linear analogue scale.

At the initial visit each side was randomly allocated active or placebo therapy. To the side receiving active therapy, an application of 0·75% 8-methoxypsoralen in a hydrophilic water/oil emulsion (Deltasoralen, Delta Laboratories, Dublin, Republic of Ireland) was applied to the entire palm and/or sole 10 min prior to exposure to UVA. The phototherapy was given using a Coast-air blacklight HF1 unit with a starting dose of 1 J/cm^2 and increasing gradually up to a maximum of 15 J/cm^2. Immediately after exposure to UVA the psoralen preparation was washed off using emulsifying ointment. To the placebo-treated side, the emulsion base only was applied for 10 min prior to exposure to the light source. A Perspex plate was inserted between the palm/sole and the UVA light source at the placebo-treated side. These procedures were carried out so that the patient was unaware which side received active treatment. All the patients had treatment three times a week over the course of 8 weeks and all completed the trial. The data on the hands and feet were analysed separately using non-parametric statistics by the Wilcoxon ranked-pairs test.

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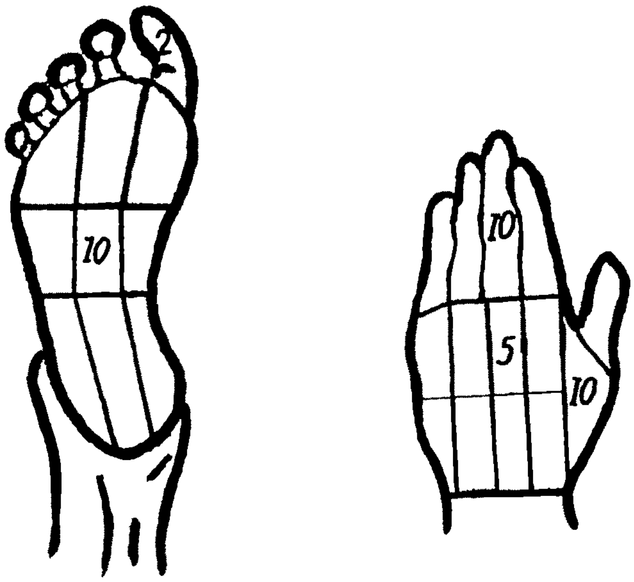

Figure 1. Assessment of percentage involvement by PPP. Foot: each toe represented 2% of the total, and the sole was divided into nine equal parts each representing 10%. Hand: the fingers, thumb and thenar eminence each represented 10% of the total, and the palm was divided into eight equal parts each representing 5%.

Table 1. Comparison of patient status of active and placebo-treated areas after topical PUVA treatment

	Active group		Placebo group	
	Feet (n=26) (%)	Hands (n=18) (%)	Feet (n=26) (%)	Hands (n=18) (%)
Cleared	0	0	0	0
Improved	18 (69)	12 (67)	18 (69)	11 (61)
Worse	4 (15)	4 (22)	5 (19)	4 (22)
Static	4 (15)	2 (11)	3 (11)	3 (17)

Results

There were no significant differences between the active and placebo-treated areas with respect to the baseline assessments.

Similar numbers of patients improved, remained static or deteriorated in the active and placebo-treated areas for both the feet and hands (Table 1). Analysis of each objective assessment showed similar results (Figs 2–5) but there were some between-group differences at 4 weeks.

Within-treatment comparisons of percentage area affected showed significant improvements from 0 at 4 weeks ($P<0{\cdot}05$) and 8 weeks ($P<0{\cdot}004$) in both the active and placebo-treated sides. Between-treatment comparisons showed significant improvements at 4 weeks for the actively treated side in both the feet ($P=0{\cdot}003$) and the hands ($P=0{\cdot}009$). However, this significance was not evident at 8 weeks (Fig. 2).

Pustule counts were reduced at 4 and 8 weeks for feet and hands. These changes were all significant except at 4 weeks in the placebo-treated hands ($P=0{\cdot}15$). There was a significant improvement between the active and placebo-treated feet at 4 weeks only ($P=0{\cdot}05$) (Fig. 3). On the hands and feet the grade according to scaling, erythema and fissuring (Fig. 4) improved significantly from 0 at 4 weeks ($P<0{\cdot}04$) and 8 weeks ($P<0{\cdot}05$), but there was no significant difference between the treatments. The patients also detected symptomatic improvement (Fig. 5) with significant changes within treatments at 4 and 8 weeks in all groups. There was also a significant between-treatment improvement at 4 weeks for the actively treated hands ($P=0{\cdot}04$) but this was not present at 8 weeks. The between-treatment comparisons are summarized in Table 2.

Side-effects were experienced by a small number of

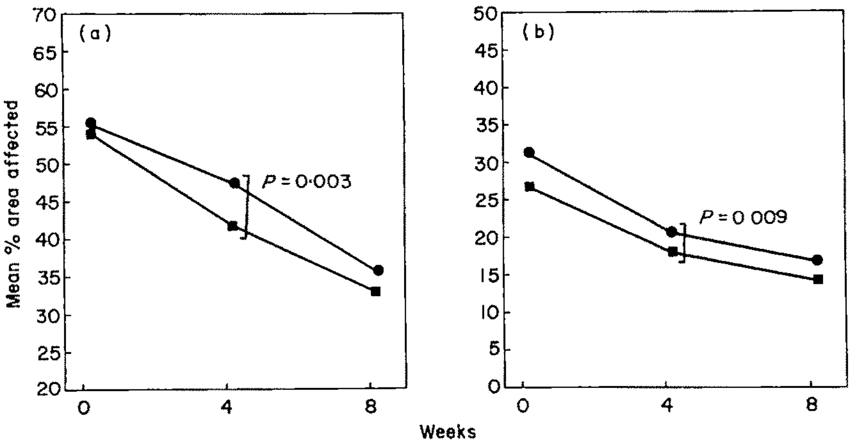

Figure 2. Mean percentage area of involvement. (a) Feet, (b) hands. ■, active; ●, placebo.

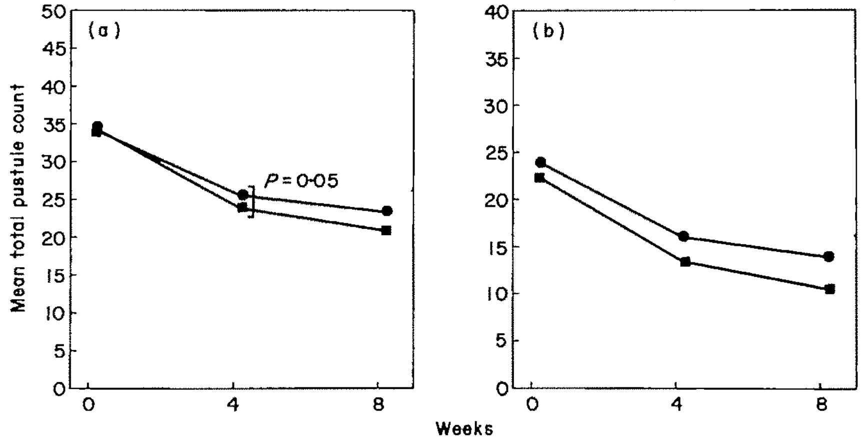

Figure 3. Mean total pustule count. (a) Feet, (b) hands. ■, active; ●, placebo.

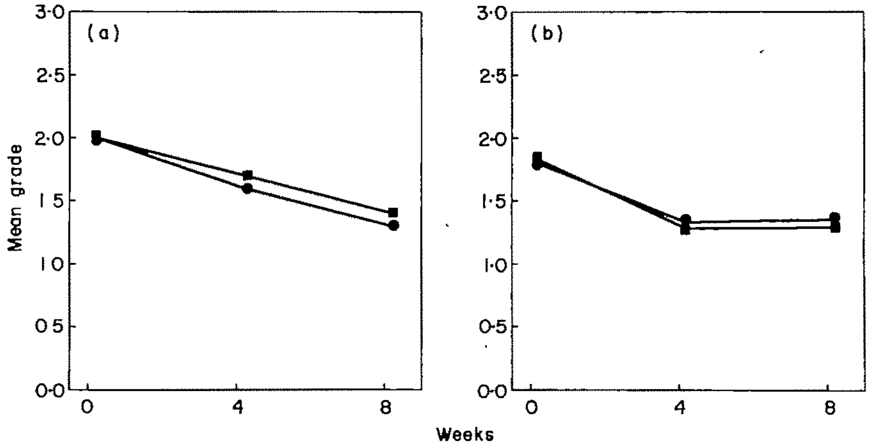

Figure 4. Mean grade for erythema, scaling and fissuring. (a) Feet, (b) hands. 0, normal or near normal skin with minimal scaling; 1, erythema with scaling only; 2, erythema, scaling and shallow fissures; 3, erythema, scaling and deep fissures. ■, active, ●, placebo.

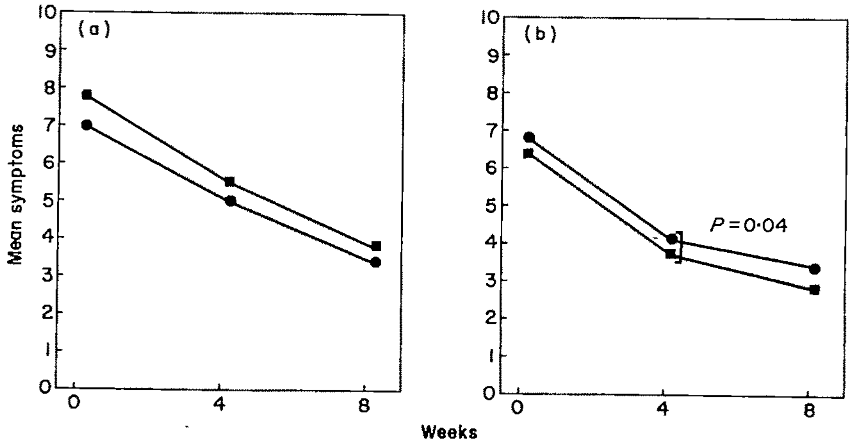

Figure 5. Mean assessment of symptoms by patients on 10 cm horizontal linear analogue scale. (a) Feet, (b) hands. ■, active; ●, placebo.

Table 2. Significant between-treatment comparisons at 4 weeks

	% Area	Pustule count	Symptoms
Feet	$P=0{\cdot}003$	$P=0{\cdot}05$	—
Hands	$P=0{\cdot}009$	—	$P=0{\cdot}04$

Table 3. Side-effects in patients receiving PUVA therapy (percentages in parentheses)

	Blistering	Pruritus	Erythema
Feet	4 (15)	3 (11)	3 (15)
Hands	3 (17)	2 (11)	2 (11)

patients receiving PUVA (Table 3). These patients were able to resume therapy after missing two consecutive treatments and restarting with a reduced dose of UVA. The mean amount of UVA in the treatment of the hands was 176 J/cm^2 and for the feet 184 J/cm^2.

Discussion

The results of this study indicate an overall improvement in both the active and placebo-treated areas of patients with PPP. A number of factors may have influenced the mean overall improvement, as it is well recognized that PPP can spontaneously relapse and remit and it is possible that some of our patients may have undergone spontaneous improvement of their disease at the time of assessment. The placebo effect of clinical trials is also well established. Thirteen of our patients resumed topical therapy at the start of the study and this may have been a further factor. Small amounts of psoralen could also be absorbed through the skin and this might have had a systemic effect and produced an improvement. However, the amount absorbed would have been extremely small and could have played little part in influencing any of the assessments.

This study has demonstrated that topical PUVA does have a limited theapeutic effect in PPP. However, the significant improvement as seen at 4 weeks was not maintained at 8 weeks and a sustained symptomatic improvement was not achieved as compared to the placebo. We would not recommend the use of topical PUVA for refractory PPP and our study contradicts previous observations made in uncontrolled studies on this disorder.[1–4] As oral psoralen photochemotherapy has been shown to be an effective treatment for PPP in a controlled trial[1] this would be more appropriate than topical psoralens for refractory PPP.

References

1 Murray D, Corbett MF, Warin AP. A controlled trial of photochemotherapy for persistent palmoplantar pustulosis. *Br J Dermatol* 1980; 102: 659–63.

2 Mizuno N, Uematsu S, Ohno M. Methoxalen and irradiation: treatment for pustulosis palmaris et plantaris. *Arch Dermatol* 1976; 112: 883–4.

3 Wilkinson JD, Ralfs IG, Harper JI, Black MM. Topical methoxsalen photochemotherapy in the treatment of palmoplantar pustulosis and psoriasis. *Acta Derm Venereol (Stockh)* 1979; 59 (Suppl): 193–8.

4 Abel EA, Goldberg LH, Farber EM. Treatment of palmoplantar psoriasis with topical methoxsalen plus long-wave ultraviolet light. *Arch Dermatol* 1980; 116: 1257–61.

5 Jansen CT, Malmiharju T. Inefficacy of topical methoxsalen plus UVA for palmoplantar pustolosis. *Acta Derm Venerol (Stockh)* 1981; 61: 354–6.

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The relationship between plasma psoralen concentration and psoralen–UVA erythema

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Summary

The plasma 8-methoxypsoralen (8-MOP) concentration was measured in 60 patients commencing psoralen photochemotherapy (PUVA). At the time of blood sampling each patient was phototested using a series of 10 exposures to UVA. The resulting erythema was measured objectively 72 h after irradiation and dose–response curves for psoralen–UVA erythema were constructed. Although the dose of 8-MOP was calculated according to body weight, patients receiving 30 mg of 8-MOP had a significantly lower mean plasma concentration than those receiving higher doses. There was no significant correlation between plasma 8-MOP concentration and minimal phototoxic dose, either estimated visually or calculated from the dose–response curves. However the slope of the dose–response curve showed significant correlation with plasma 8-MOP concentration. The variation between patients in the rate of increase of the erythemal response, but not the variation in threshold sensitivity, can be explained by differences in plasma psoralen concentration.

In psoralen photochemotherapy (PUVA) the main factor which limits the dose of UVA radiation that can be given at each treatment is the development of erythema, which is maximal 48–72 h after irradiation.[1] Although the dose of UVA used at the start of a course of treatment is usually chosen according to the sun-reactive skin type of the patient,[1,2] we and others have shown that the marked variation in erythemal sensitivity to PUVA between patients is not related to skin type.[3,4] The plasma concentration of 8-methoxypsoralen (8-MOP) after an oral dose determined according to body weight is also known to show considerable variation between patients.[5,6] In order to study further the variation in erythemal sensitivity to PUVA we have measured simultaneously the plasma psoralen concentration and psoralen–UVA erythemal response in patients at the start of PUVA treatment.

Methods

Patients

We studied consecutive patients commencing treatment with PUVA, excluding those who had received phototherapy within the previous 6 months or who had insufficient normal skin for phototesting. Of the 60 patients studied, 54 had psoriasis and the remaining six patients were receiving treatment for pre-mycotic eruptions or eczema. Clinically normal skin on the flexor aspect of the forearm was used for phototesting and the sun-reactive skin type of each patient was recorded.

8-methoxypsoralen in a crystalline form (Promedica, Levallois-Perret, France) was given orally at a dose of 0·6 mg/kg (calculated to the nearest 10 mg) 2 h before phototesting. No dietary advice was given to the patients.

Phototesting procedure

Neutral density filters of varying thickness were mounted over 1-cm diameter apertures in a UVA opaque plastic template which was secured to the flexor aspect of the forearm. The remaining skin of the forearm and hand was protected from UVA exposure using a tightly woven cotton sleeve. The whole forearm was then exposed to radiation from 15 standard PUVA fluorescent lamps (Philips TL 40W/09R) mounted in a cylindrical configuration resulting in a uniform irradiance at the skin surface of 20 mW/cm^2.[3] As the neutral density filters alter the intensity of the radiation and not the spectrum, a single exposure of 30 J/cm^2 to the template-protected arm thus resulted in 10 skin sites being exposed simultaneously to a series UVA doses ranging from 0·8 to 30 J/cm^2. UVA transmission through the filtered template was measured using a TL 40W/09R lamp in conjunction with a spectroradiometer (model 742; Optronic Labs Inc, Orlando, FL, U.S.A.) and the irradiance of the UVA lamps used for phototesting was measured using a radiometer (IL 700; International Light, U.S.A.).

Measurement of plasma psoralen concentration

A 10-ml sample of venous blood taken at the commencement of phototesting was anticoagulated using preservative-free heparin before extraction and analysis using a modification of a previously described technique.[7] In brief, plasma samples were passed through Sep-Pak-C18 solid phase-extraction cartridges (Waters-Millipore, Cheshire, U.K.). An additional washing step using 10-ml of distilled water introduced after the plasma was applied to the cartridge was found to result in a cleaner elution of 8-MOP, and to reduce particulate matter in the extract. The 8-MOP collected from the cartridge was analysed by reversed-phase HPLC using a u Bondpak phenyl chromatography column with Waters-Millipore (Cheshire) equipment.

In 14 patients the plasma psoralen levels were measured at two subsequent attendances for PUVA treatment, not necessarily at the same time of day as the first attendance but still 2 h after ingestion of the 8-MOP tablets.

Measurement of erythema

A visual assessment of the minimum dose of UVA required to produce just detectable erythema (minimum phototoxic dose (MPD)), was made 72 h after irradiation in all patients. Objective measurements of the intensity of erythema at each site were made at the same time using a reflectance instrument which obtained an erythema index related to the blood content of the superficial dermis.[8] Triplicate measurements were made and the vasodilatation due to the radiation was expressed as the difference between the mean erythema index at each test site and the mean erythema index on adjacent unirradiated skin.[9]

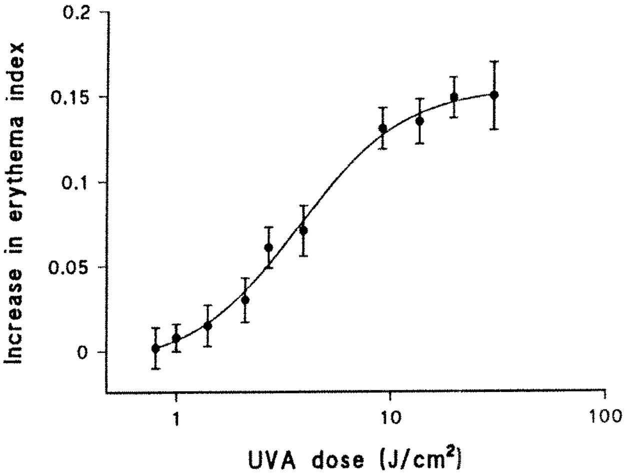

Figure 1. An example of the dose–response curve for PUVA erythema in one patient. Each point represents the mean (±SD) increase in erythema index over background measurements. The solid line through the data points is the logit function obtained by regression analysis.

For every patient the increase in erythema index measured at each test site was plotted against the logarithm of the UVA dose. An iterative, least squares, linear regression technique was used to fit a logit function to the data obtained for each patient (Fig. 1). The maximum slope of this function was calculated, and also the dose of UVA ($D_{0\cdot025}$) required to achieve an increase in erythema index of 0·025 (equivalent to a just perceptible erythema).

Results

Patients

A total of 60 patients, 35 male and 25 female with a median age of 35 years (range 11–79 years) were studied. Five patients were of skin type I, 23 of skin type II, 22 of skin type III and 10 of skin type IV.

PUVA erythemal response

Two of the patients failed to return for measurement of the phototest responses, and one developed extensive eczema over the test sites, precluding their measurement. Of the remaining 57 patients, nine showed no erythemal response at the test sites, even to the highest UVA dose of 30 J/cm^2. For subsequent statistical analysis the MPD and $D_{0\cdot025}$ for these patients were judged to be greater than 30 J/cm^2. Sufficient data points to construct a dose–response curve (Fig. 1) for PUVA erythema were available in 47 patients.

The Kruskal–Wallis test showed no significant difference between skin types as regards erythemal sensitivity to PUVA, assessed either as the MPD or measured $D_{0\cdot025}$ (Table 1).

Plasma psoralen concentration

The plasma concentration of 8-MOP ranged from undetectable (<10 ng/ml) to 890 ng/ml, and values for the 60 patients showed a positive-skewed distribution (Fig. 2). The mean coefficient of variation obtained from

Table 1. The MPD (J/cm^2) and $D_{0\,025}$ (J/cm^2) according to sun-reactive skin type

Sun-reactive skin type							
I		II		III		IV	
MPD	$D_{0\,025}$	MPD	$D_{0\,025}$	MPD	$D_{0\,025}$	MPD	$D_{0\,025}$
>30	>30	2·7	2·6	2·7	2·2	>30	<30
0 8	1·3	>30	>30	3·9	1·4	2 4	2·3
2·7	1·5	>30	>30	2·1	1·8	9·1	12·1
1·0	0·8	2·7	1·3	>30	>30	3·9	3·0
9·1	9·0	2·7	2·4	3·9	1·9	2·7	2·2
		5·0	4·9	3·9	1·7	>30	>30
		2·7	2·3	3·5	3·2	9·1	10·0
		3·9	3·3	2·4	1·8	>30	>30
		3·9	2·9	2·7	1·4	1·4	0·9
		2·7	1·7	>30	>30	2·0	0·8
		2·7	1·8	3·9	5·0		
		7 1	10·0	2·7	1·5		
		7·1	6·2	10·2	11·7		
		2·7	0·6	1 0	1·1		
		1·1	2·9	3·9	1·2		
		6·0	5·9	9·1	8·8		
		2·7	0·5	2·7	3·7		
		2·7	1·7	1·4	3·7		
		2·6	3·7	>30	>30		
		1 4	1·5	1·4	1·6		
		4·5	5·7				
		1·4	2·2				

triplicate assays of aliquots of plasma from each patient was 4·1%. Analysis of variance of log-transformed data showed a significant difference (F-ratio 6·7; $P<0·001$) in plasma concentration depending on the dose of 8-MOP (Fig. 3). The mean plasma concentration in patients taking an 8-MOP dose of 30 mg was significantly lower ($P<0·01$, t-test) than that for patients taking a higher dose, but the mean concentration was otherwise independent of the dose administered.

The results of plasma psoralen measurements in the 14 patients from whom blood samples were obtained on three different treatment days are shown in Table 2.

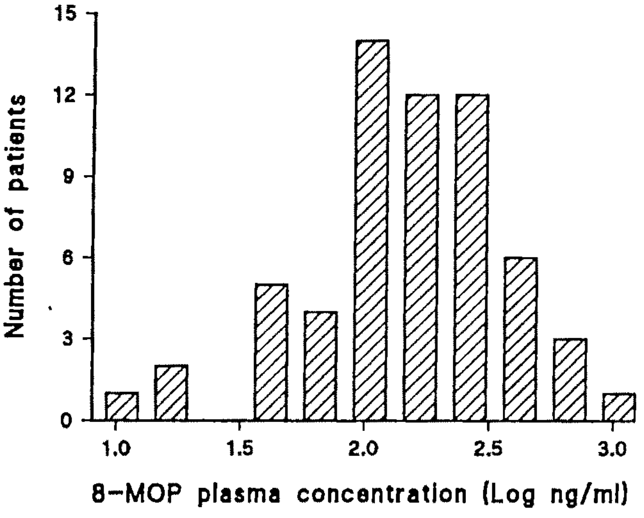

Figure 2. The distribution of plasma 8-methoxypsoralen concentration in 60 patients.

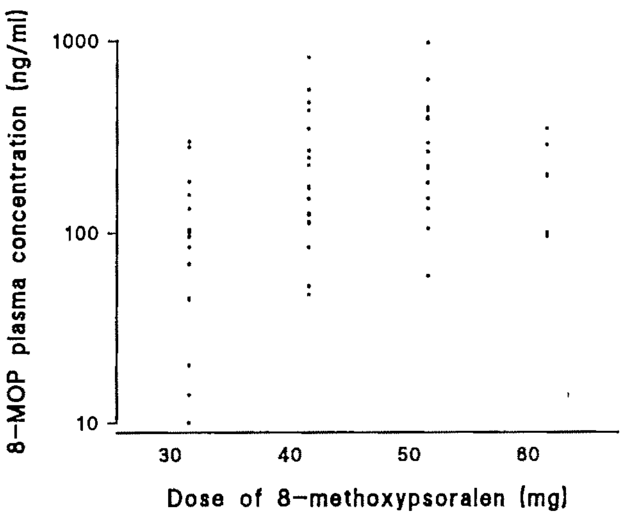

Figure 3. The variation in 8-methoxypsoralen concentration according to dose.

Table 2. The variation in plasma 8-methoxypsoralen concentration on three different treatment days in 14 patients

	Plasma 8-MOP concentration (ng/ml)		
Patient number	Sample 1	Sample 2	Sample 3
1	20	21	32
2	314	238	84
3	170	111	96
4	299	250	286
5	56	20	182
6	72	81	14
7	320	329	348
8	120	52	199
9	52	662	870
10	264	322	112
11	351	228	264
12	177	412	124
13	395	126	68
14	253	328	246

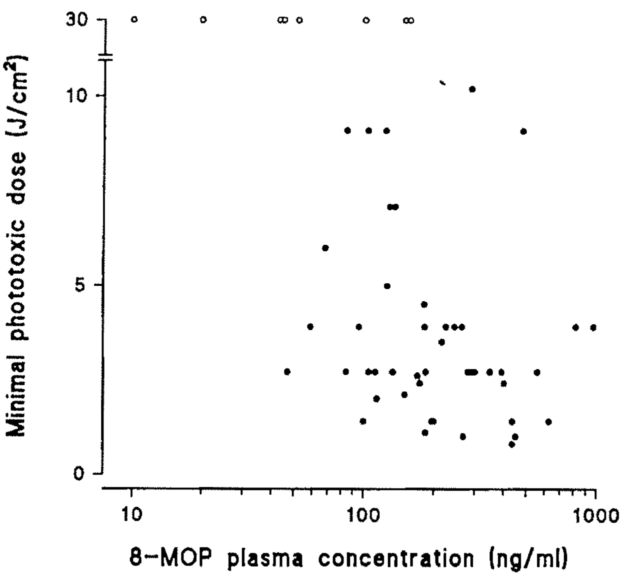

Figure 4. The relationship between plasma 8-methoxypsoralen concentration and minimal phototoxic dose. Patients in whom the highest dose of radiation (30 J/cm^2) failed to achieve erythema are represented by open circles.

Analysis of variance on log-transformed concentrations showed significantly greater between-patient than within-patient variation (*F*-ratio 3·5; $P<0·05$).

The lowest 8-MOP concentration associated with the development of skin erythema was 44 ng/ml; six out of the nine patients with no detectable erythema had plasma 8-MOP concentrations below that level. Apart from this threshold effect there was no significant correlation betweeen plasma 8-MOP concentration and PUVA erythemal sensitivity, assessed either as the MPD (Fig. 4) or measured $D_{0.025}$ (Fig. 5). However, plasma 8-MOP concentration and the maximum slope of the PUVA erythemal dose–response curve showed significant correlation (Spearman rank correlation coefficient 0·49; $P<0·001$) (Fig. 6).

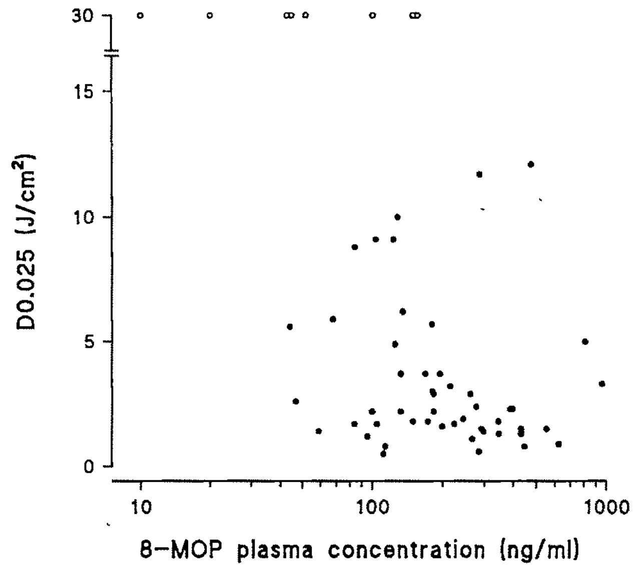

Figure 5. The relationship between plasma 8-methoxypsoralen concentration and $D_{0.025}$. Patients in whom the highest dose of radiation (30 J/cm^2) failed to achieve erythema are represented by open circles.

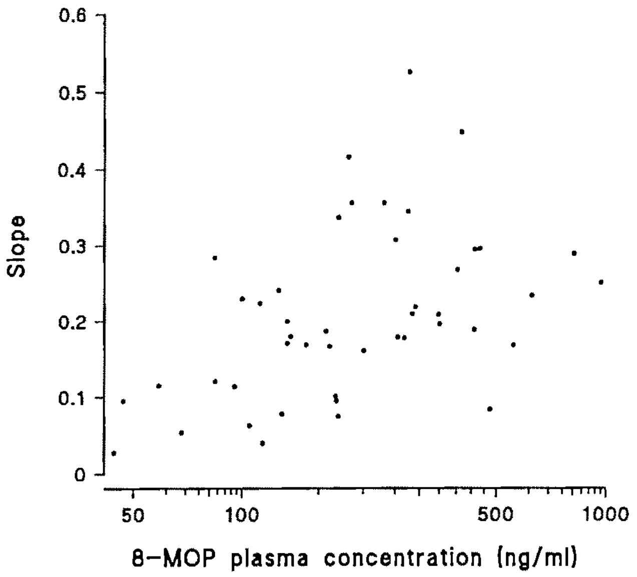

Figure 6. The relationship between plasma 8-methoxypsoralen concentration and maximum slope of the PUVA erythemal dose–response curve. Spearman rank correlation coefficient$=0·49$ ($P<0·001$).

Discussion

We have shown that the rate of increase but not the threshold of the PUVA erythemal response corresponds to plasma 8-MOP concentration.

A novel method of phototesting using a series of neutral density filters allowed simultaneous exposure of 10 skin sites on the forearm to different UVA doses. The intensity of erythema at each of the sites was measured objectively 72 h after irradiation, and a logit function was fitted to the data obtained for each patient to result in a sigmoid dose–response curve. The maximum slope of each curve was calculated, and also the dose of UVA radiation ($D_{0\cdot025}$) that would be required to achieve an increase in erythema index of 0·025, a value corresponding to just detectable erythema.

We have confirmed our previous findings[3] and also those of other workers[4] that there is no significant relationship between sun-reactive skin type and erythemal sensitivity to PUVA assessed as the MPD. The value of $D_{0\cdot025}$, calculated from the full dose–response data in each patient, should be a more accurate indication of sensitivity than the visually observed MPD which, like any minimal erythema measurement, is influenced by the magnitude of the UVA dose increments and also the exact degree of erythema observed.[10] Even with sensitivity expressed as the calculated $D_{0\cdot025}$ (Table 1), or other doses such as the $D_{0\cdot05}$ (data not shown), there is still no significant relationship with skin type.

As found in previous studies,[5,6] plasma 8-MOP concentration showed great variability between patients. Under conditions of strict control of fluid and food intake it has been shown that 8-MOP serum concentrations show greater between-patient than within-patient variation.[11] We have extended this observation by making repeated measurements of 2-h MOP plasma concentration on different treatment days in 14 patients, without imposing dietary restrictions. Our results (Table 2) show that within-patient variability was still less than between patient variability under conditions of normal clinical practice. Our finding that patients given 30 mg of 8-MOP had significantly lower plasma concentrations than those receiving higher doses (Fig. 3) even though this dose was determined by body weight, suggests that determination of 8-MOP dose by this method is not ideal. This may have relevance for treatment as some patients with a poor response to therapy are known to have lower than average plasma psoralen concentrations.[12]

In nine patients the phototest failed to produce an erythematous response, even at sites exposed to the highest dose of UVA radiation (30 J/cm^2). Goldstein *et al.*[6] found that a plasma 8-MOP concentration of between 30 and 50 ng/ml was required to achieve a phototoxic erythematous response. In keeping with this, six of our non-responding patients had plasma 8-MOP concentrations less than 50 ng/ml, and the lowest concentration accompanied by an erythematous response was 44 ng/ml. Apart from this 'threshold' effect, there was no correlation between plasma 8-MOP concentration and erythemal sensitivity to PUVA expressed either as the MPD (Fig. 4) or calculated $D_{0\cdot025}$ (Fig. 5). This is in contrast to Herfst and De Wolff[11] who found a highly significant correlation between the mean maximum blood psoralen concentration (when measured at half-hourly intervals) and the mean MPD (with irradiation 2 h after psoralen administration) of readings measured on three occasions in 18 patients. Although we studied a much larger number of patients we were unable to confirm these findings and the reason for this is uncertain. One difference between our study and that of Herfst and De Wolff[11] is that we only measured plasma concentration of 8-MOP at a single time point (the time of irradiation) 2 h after oral administration. Variations in plasma concentration of 8-MOP after the time of irradiation are unlikely to be of relevance in psoralen phototoxicity. Similarly, although our measurement may not have coincided with the maximum 8-MOP plasma concentration this cannot explain the different results, as for individual patients the MPD has been shown to vary inversely with 8-MOP blood concentrations 1–5 h after the oral dose of psoralen.[13]

In our study the slope of the dose–response curve showed a highly significant correlation with plasma 8-MOP concentration (Fig. 6). The relationship between the slope of the PUVA erythema dose–response curve and therapeutic effect now requires investigation.

The cause of the large variation in erythemal sensitivity to PUVA between patients remains to be established. It is not related to skin type, UVB erythemal sensitivity,[3] or to 8-MOP plasma concentration provided that a concentration greater than 44 ng/ml is achieved.

Acknowledgment

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References

1 Wolff K, Gschnait F, Honigsmann H *et al.* Phototesting and dosimetry for photochemotherapy. *Br J Dermatol* 1977; **96:** 1–14.

2 Farr PM, Diffey BL. PUVA treatment of psoriasis in the United Kingdom. *Br J Dermatol* 1991; **124:** 365–7.

3 Cox NH, Farr PM, Diffey BL. A comparison of the dose-response relationship for psoralen–UVA erythema and UVB erythema. *Arch Dermatol* 1989; **125:** 1653–7.
4 Jansen CT. Self-reported skin type and reactivity to UVB, UVA and PUVA irradiation. *Photodermatology* 1989; **6:** 234–6.
5 Ljunggren B, Carter DM, Albert J, Reid T. Plasma levels of 8-methoxypsoralen determined by high-pressure liquid chromatography in psoriatic patients ingesting drug from two manufacturers. *J Invest Dermatol* 1980; **74:** 59–62.
6 Goldstein DP, Carter DM, Ljunggren B, Burkholder J. Minimal phototoxic doses and 8-MOP levels in PUVA patients. *J Invest Dermatol* 1982; **78:** 429–33.
7 Gasparro FP, Battista BS, Song J, Edelson RLM. Rapid and sensitive analysis of 8-methoxypsoralen in plasma. *J Invest Dermatol* 1988; **90:** 234–6.
8 Diffey BL, Oliver RJ, Farr PM. A portable instrument for quantifying erythema induced by ultraviolet radiation. *Br J Dermatol* 1984; **111:** 663–72.
9 Farr PM, Diffey BL. Quantitative studies on cutaneous erythema induced by ultraviolet radiation. *Br J Dermatol* 1984; **111:** 673–82.
10 Farr PM, Diffey BL. The erythemal response of human skin to ultraviolet radiation. *Br J Dermatol* 1985; **113:** 65–76.
11 Herfst MJ, De Wolff FA. Intraindividual and interindividual variability in 8-methoxypsoralen kinetics and effect in psoriatic patients. *Clin Pharmacol Ther* 1983; **34:** 117–25.
12 Wagner G, Hofmann C, Busch U *et al.* 8-MOP plasma levels in PUVA problem cases with psoriasis. *Br J Dermatol* 1979; **101:** 285–92.
13 Swanbeck G, Ehrsson H, Ehrnebo M, Wallin I. Serum concentration and phototoxic effect of methoxsalen in patients with psoriasis. *Clin Pharmacol Ther* 1979; **25:** 478–80.

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Naevocytic naevi with vascular-like spaces

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Summary Fourteen cases of naevocytic naevi with anastomising lacunae suggestive of vascular spaces are reported. The cells lining these lacunae were consistent with naevus cells, being positive for vimentin and S100 protein and negative for factor VIII-related antigen and *Ulex europaeus* I. The cells were not surrounded by laminin or type IV collagen. We suggest the formation of these vascular-like spaces may be due to defective production or increased degradation of components of the basement membrane with a consequent lack of cohesion.

The diagnosis of naevocytic naevus is not difficult although sometimes these lesions show unusual histological features such as the deposition of amyloid,[1] bone formation,[2] presence of elastic fibres[3] and psammomatous bodies.[4] In 1972 Sagebiel[5] reported the presence of endothelial-lined spaces in naevocytic naevi which he described as being similar to vascular channels, but lined with naevus cells. He suggested that these changes were caused by the injection of anaesthetic and with consequent tissue shrinkage. In a study of unusual features of naevocellular naevi, Weedon[6] interpreted these spaces as being histological artefacts.

The purpose of this study was to investigate, using histochemical and immunohistochemical methods, a series of benign naevocytic naevi that had anastomosing vascular-like spaces in order to determine further their nature.

Methods

One-hundred and seventy-five unselected naevocytic naevi were reviewed and of these only those showing vascular-like spaces were examined, using histochemical and immunohistochemical methods.

Histochemistry

The sections were dewaxed in xylene and endogenous peroxidase activity blocked using a 0·3% solution of hydrogen peroxide in methanol for 30 min. The sections were then rinsed with ethanol and treated with pronase at 37°C for 15 min. They were then incubated sequentially at room temperature with bovine serum albumin for 10 min, *Ulex europaeus* agglutinin I (Vector, Burlingame, U.S.A.) in phosphate-buffered solution (PBS) supplemented with Ca^{2+} and Mg^{2+} for 2 h, and then for 45 min with avidin–biotin complex. Three washes with PBS were performed betwen the sequential steps and then the chromogen, 0·03% 3,3′diaminobenzidine in PBS with 0·01% hydrogen peroxide, was placed on the sections for 3 min and these were counterstained with haematoxylin.

Immunohistochemistry

The ABC method of Hsu *et al.*[7] modified by the use of streptavidin–biotin peroxidase complex (Ortho Diagnostic Systems, Milan, Italy) was used on the sections. The primary antisera used are listed in Table 1. As control sections those with known positivity and negativity were included with each batch of slides.

Results

Vascular-like spaces were found in 14 (8%) naevocytic naevi obtained from 10 patients. The clinicopathological features of these naevi are summarized in Table 2. Eight of the patients were female and two were male, and their age range was 23–54 years (mean 31·3 years). All the lesions were described as being polyploid or dome-shaped.

Histology

Seven of the cases were intradermal naevi and seven compound naevi. In five patients, the naevocytic naevi were multiple and in two of these cases all of the removed

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Table 1. Antisera used for immunohistochemical analysis

Antisera	M/P	Source	Dilution
S100 protein	P	Dakopatts	1:3600
Vimentin	M	Dakopatts	1:200
Factor VIII	P	Dakopatts	1:2500
Laminin	P	Dakopatts	1:500
Collagen IV	M	Dr H.Battifora Duarte, U.S.A.	1:20

M, monoclonal; P, polyclonal.

Table 2. Clinicopathological details of patients with naevocytic naevi with vascular-like spaces

Case	Sex	Age (y)	Site	No. of lesions selected
1	F	25	Back	3 (3)
2	F	23	Back	3 (3)
3	F	23	Face	1
4	F	35	Back	1 (2)
5	M	28	Back	1
6	F	37	Shoulder	1 (3)
7	F	27	Back	1 (2)
8	F	24	Abdomen	1
9	M	37	Arm	1
10	F	54	Breast	1

(n), number of naevocytic naevi removed at the same time from the same patient.

lesions showed these vascular-like spaces. The extent of the vascular-like spaces varied between cases, although in most (Cases 2–9) they were only present focally. In one case (Case 1) they appeared widely distributed throughout the lesion. The vascular-like spaces were mostly located in the mid-dermis and near the dermal–epidermal junction. At low power, they mimicked a vascular lesion by appearing as cell-lined empty spaces that had their long axes often perpendicular to the epidermis (Fig. 1). The spaces showed an anastomosing pattern (Figs 2 and 3) and appeared to be bordered by cells that had an eosinophilic cytoplasm and dark staining nuclei (Fig. 3). Papillary-like structures projected into the empty spaces and were similar to those seen in Masson's haemangioendothelioma (Fig. 4). Some of the cells appeared to 'float' freely in the empty spaces.

Immunohistochemistry

The naevocytic cells and the cells lining the vascular-like

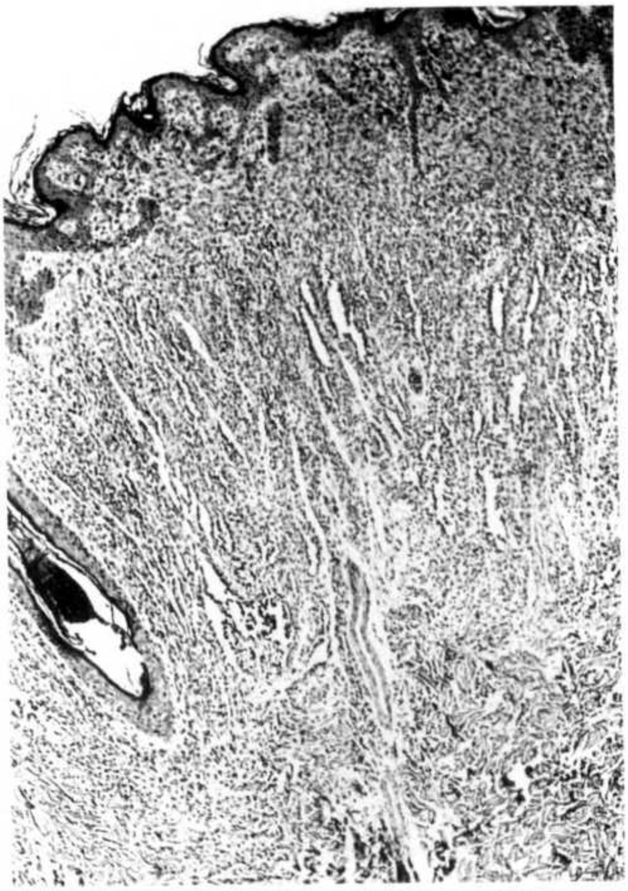

Figure 1. Intradermal naevocytic naevus showing vascular-like spaces with long axes perpendicular to the epidermis (haematoxylin and eosin, × 50).

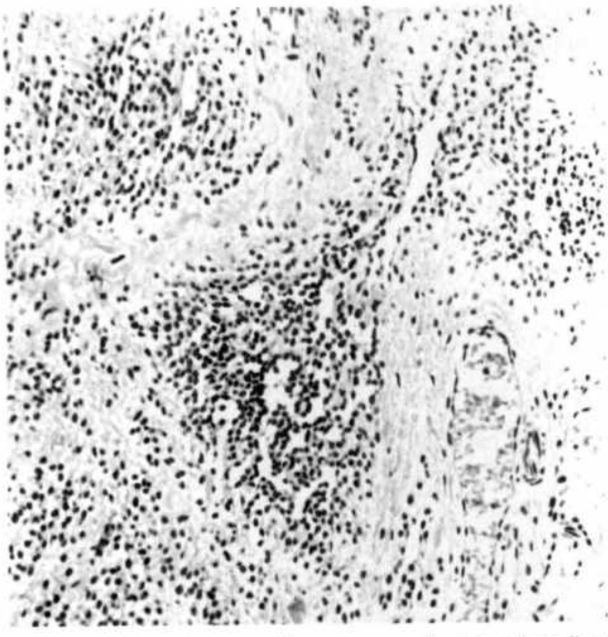

Figure 2. Anastomosing empty lacunar spaces dissecting the collagenous stroma (haematoxylin and eosin, × 65).

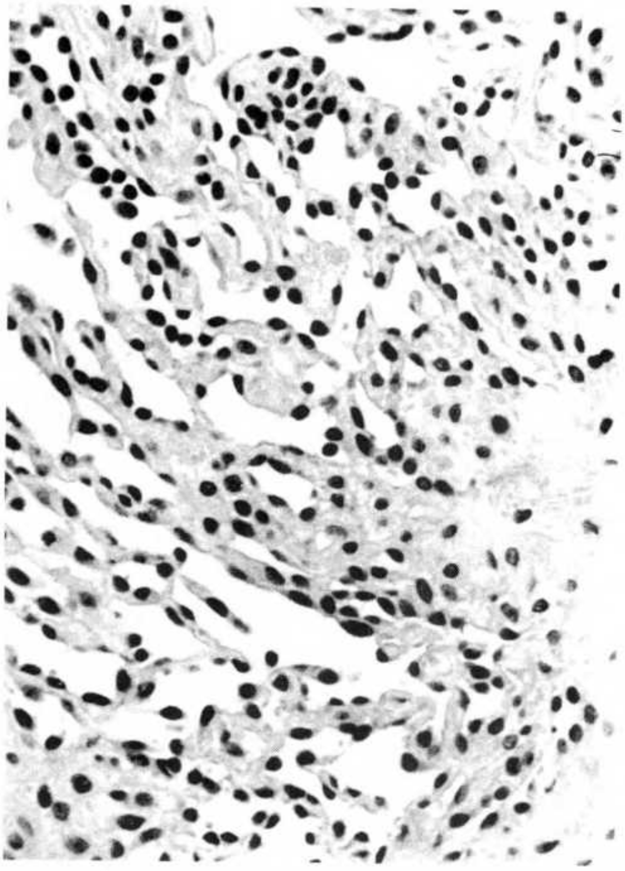

Figure 3. Anastomosing spaces bordered by cells with dark nuclei (haematoxylin and eosin, × 80).

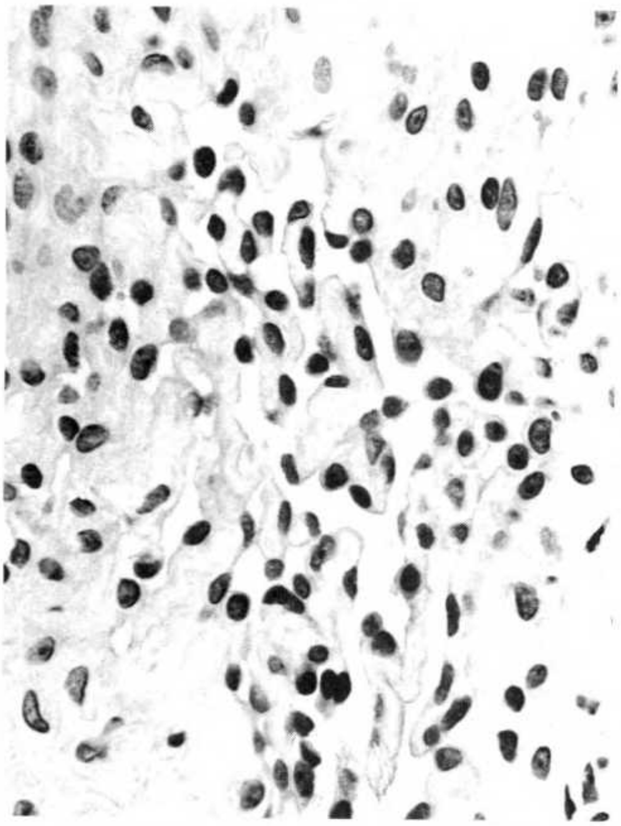

Figure 4. Small micropapillary structures reminiscent of a benign endothelial proliferation within the anastomosing lacunae (haematoxylin and eosin, × 250).

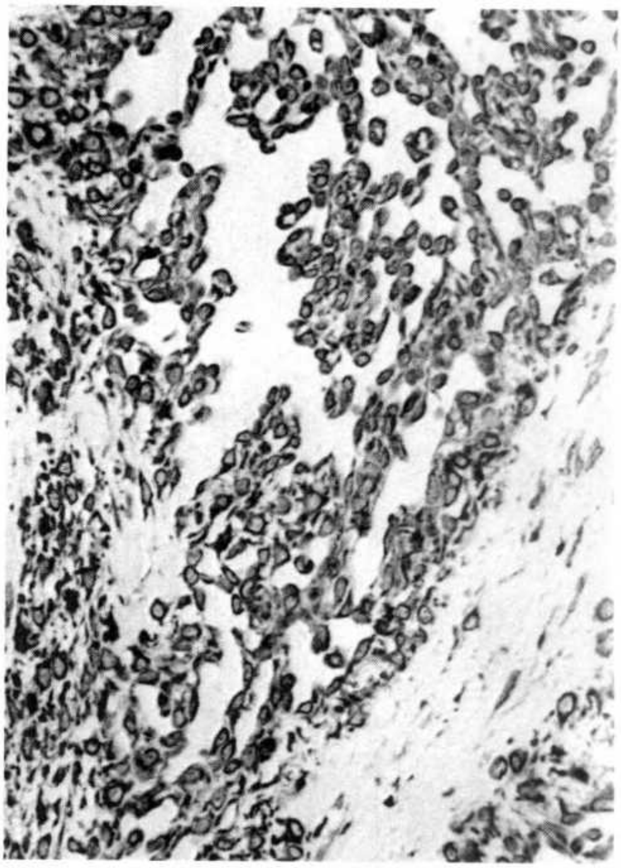

Figure 5. Strong immunoreactivity with S100 protein antiserum in the cells lining the vascular-like spaces. (Streptavidin–biotin peroxidase complex, × 125).

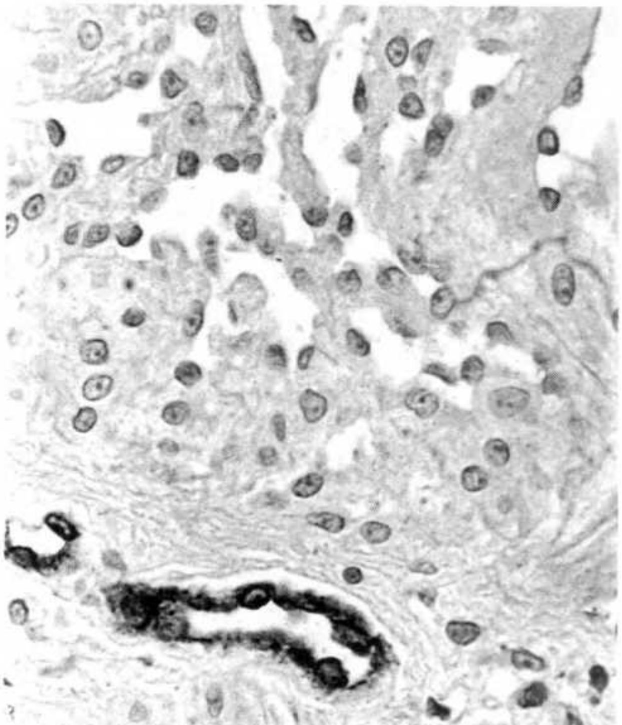

Figure 6. Cells lining the vascular-like spaces are negative for factor-VIII related antigen. Endothelial cells are positive. (Streptavidin–biotin peroxidase complex, × 350).

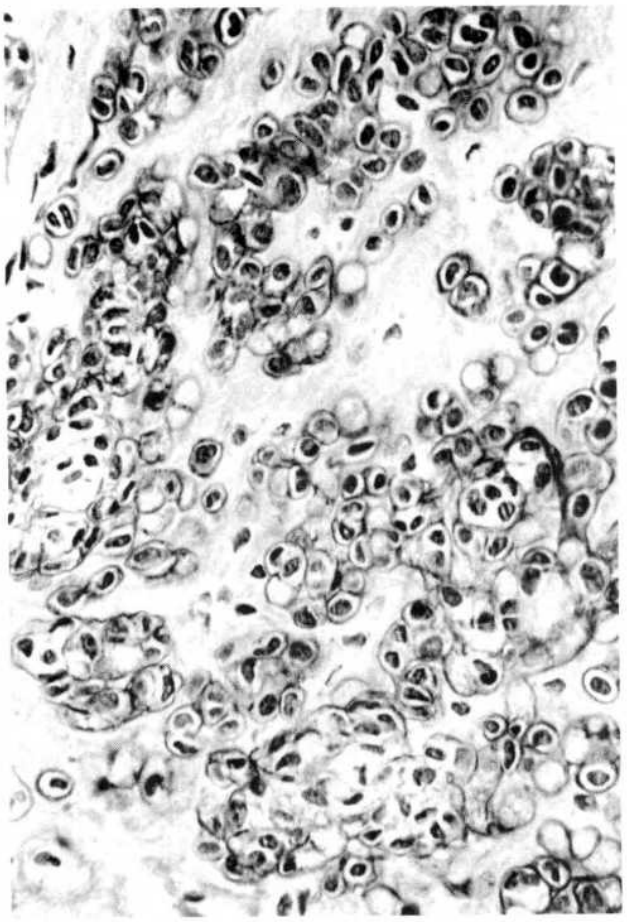

Figure 7. Immunoreactivity with laminin antiserum at the boundary of each naevocytic cell. (Streptavidin–biotin peroxidase complex, × 250).

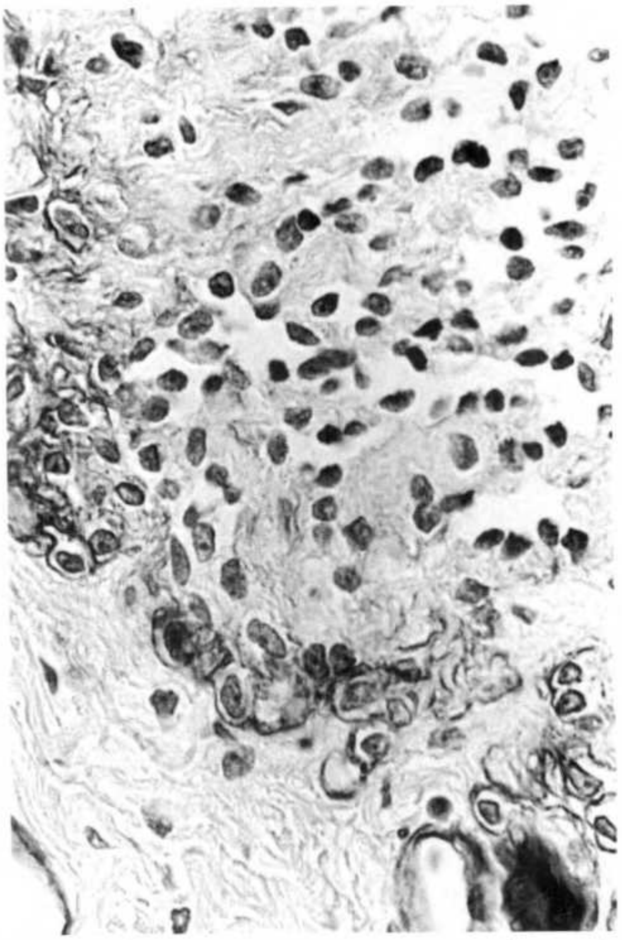

Figure 8. Laminin immunoreactivity is not seen around naevocytic cells lining the vascular-like spaces. (Streptavidin–biotin peroxidase complex, × 250).

spaces were strongly positive for both vimentin and S100 protein (Fig. 5). A similar positivity was found in the cytoplasm of the cells that were 'floating' in the empty spaces and those lining the papillary-like structures. These cells were negative for factor VIII-related antibody (Fig. 6) and the lectin *Ulex europaeus* I. When antibodies to type IV collagen and laminin were used, there was a strong positivity at the cytoplasmic borders of the naevocytic cells both in the dermis and near the dermal–epidermal junction (Fig. 7), but the cells lining the vascular-like spaces were either negative or only focally positive (Fig. 8).

Discussion

Of the 175 naevocytic naevi that were examined, 14 of them showed vascular-like spaces. Five of the patients had more than one naevus removed from the same body site but in only two of these cases were the vascular-like spaces seen in the lesions. The cells lining the vascular-like spaces appeared to be consistently positive for S100 protein and vimentin, markers of naevocytic cells,[8–11] and were consistently negative for the lectin *Ulex europaeus* I and factor-VIII-related antigen, markers for endothelial cells.[12–15]

The view that the vascular-like spaces in these naevi are histological artefacts is in our opinion incorrect, particularly as the injections of the local anaesthetic are usually performed away from the lesions. Most naevi surgically removed are done under local anaesthesia and it is only rarely that the vascular-like spaces in them are apparent. Also, Case 10 was a naevocytic naevus that was on a breast removed for carcinoma and under general anaesthesia.

The basal lamina (BL) is a complex extracellular matrix that separates epithelial, mesothelial and endothelial cells from connective tissue stroma.[16] It consists of both intrinsic and extrinsic components.[17] Laminin and type IV collagen are two intrinsic components of BL[16] which are produced by several cells, including naevocytic cells.[18,19] In the present study the naevocytic cells showed positive pericytoplasmic staining with type IV collagen and with an antiserum to laminin. However the cells that lined these vascular-like spaces suggest a defective production or an increased degradation of BL constituents. Perhaps this defect is responsible for the lack of cohesion and results in the formation of vascular-like spaces.

Acknowledgment

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References

1 MacDonald DM, Black MM. Secondary localized cutaneous amyloidosis in melanocytic naevi. *Br J Dermatol* 1980; **103**: 553–6.

2 Duperrat P, Monfort G. L'osteo-naevus de Nanta. *Bull Soc Derm et Syphil* 1958; **4**: 514–7.

3 Mehregan AH, Starrico RG. Elastic fibers in pigmented nevi. *J Invest Dermatol* 1962; **38**: 271–6.

4 Weitzner S. Intradermal nevus with psammoma body formation. *Arch Dermatol* 1968; **98**: 287–9.

5 Sagebiel RW. Histologic artifacts of benign pigmented nevi. *Arch Dermatol* 1972; **106**: 691–3.

6 Weedon D. Unusual features of nevocellular nevi. *J Cut Pathol* 1982; **9**: 284–92.

7 Hsu SM, Raine LO, Fanger H. The use of avidin–biotin peroxidase complex (ABC) in immunoperoxidase technique: a comparison between ABC and unlabelled antibody (PAP) procedures. *J Histochem Cytochem* 1981; **29**: 577–80.

8 Nakajima T, Watanabe S, Sato Y *et al.* Immunohistochemical demonstration of S 100 protein in malignant melanoma and pigmented nevus and its diagnostic application. *Cancer* 1982; **50**: 912–18.

9 Miettinen M, Lehto UP, Virtanen I. Presence of fibroblast-type intermediate filaments in pigmented nevi and malignant melanomas. *J Cutan Pathol* 1983; **10**: 188–92.

10 Yambe H, Ishii Y, Tabuchi K *et al.* Detection of S 100 protein in melanocytic and neurogenic cutaneous tumors. *Arch Dermatol Res* 1984; **276**: 160–4.

11 Paul E, Wen D-R, Cochran AJ. Variations in S 100 protein expression in naevocellular naevi may be related to metabolic activity. *Br J Dermatol* 1987; **116**: 371–8.

12 Sehested M, Hou-Jensen I. Factor VIII-related antigen as endothelial marker in benign and malignant disease. *Virchows Arch [A]* 1981; **370**: 323–32.

13 McComb RD, Trevor RJ, Pizzo SV *et al.* Specificity and sensitivity of immunohistochemical detection of factor VIII/von Willebrand factor antigen in formalin-fixed paraffin-embedded tissue. *J Histochem Cytochem* 1982; **30**: 371–7.

14 Holthofer H, Virtanen I, Kariniemi A-L *et al. Ulex Europaeus* I lectin as a marker for vascular endothelium in human tissues. *Lab Invest* 1982; **47**: 60–6.

15 Walker RA. *Ulex Europaeus* I-peroxidase as marker of vascular endothelium: its application in routine histopathology. *J Pathol* 1985; **146**: 123–7.

16 Lee AK. Basement membrane and endothelial antigens: their role in evaluation of tumor invasion and metastasis. In: *Advances in Immunohistochemistry*. Ronald A. De Lellis. New York: Raven Press, 1988; 363–93.

17 Martinez-Hernandez A, Amenta PS. The basement membrane in pathology. *Lab Invest* 1983; **48**: 656–77.

18 Natali PG, Nicotra M, Belloci M *et al.* Distribution of lamin and collagen type-IV in benign and malignant lesions of melanocytic origin. *Int J Cancer* 1985; **35**: 461–7.

19 Yaar M, Woodley TD, Gilchrest BA. Human nevocellular nevus cells are surrounded by basement membrane components. Immunohistologic studies of human nevus cells and melanocytes *in vivo* and *in vitro*. *Lab Invest* 1988; **58**: 157–62.

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In-vitro and *in-vivo* studies of human axillary odour and the cutaneous microflora

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Summary

The axillary microflora of 34 male subjects were studied in relation to their underarm odour intensity. The predominant groups of micro-organisms were aerobic coryneforms, *Micrococcaceae* and propionibacteria. There was no competition for habitat between these groups (Fisher's exact test $P > 0{\cdot}05$). There was an association between the population density of aerobic coryneforms and the intensity of odour (Spearman, $P = 0{\cdot}001$). Dominance of aerobic coryneforms within the axillary microflora was associated with high odour intensity (χ^2, $P = 0{\cdot}005$). An *in-vitro* odour model was developed using a diethyl ether extract of axillary skin incubated with test bacteria. Underarm odour was produced exclusively by aerobic coryneform bacteria. Of aerobic coryneforms, 71·4% were odour producers and these were identified as *Corynebacterium xerosis*.

Human odour has been of great concern to society. The feet, the groin, the hair, the breath and the general surface of the body all have characteristic odours, but the axillae generally produce the most powerful and easily recognizable odour. The evidence indicates that it is an interaction of the axillary microflora with apocrine sweat which gives rise to the odour. An association was found between high microbial population densities of the axilla and odour, and high-odour people had a greater proportion of lipophilic and non-lipophilic coryneforms in their axillary microflora than low-odour people.[1] Using an *in-vivo* model of aprocine sweat on the occluded volar forearm inoculated with various axillary bacterial isolates, it was shown that only the coryneform bacteria produced the typical axillary odour.[1] The latter result has been confirmed by Labows *et al.*[2] Further evidence implicating bacterial microflora in underarm odour is its reduction when antimicrobials are used as deodorants.[3,4]

Numerous odorous substances have been detected and quantified in human axillary secretions, including fatty acids of chain length C_2–C_{16}[5] and some 16-androstene steroids.[6] The presence of the urine-smelling 5α-androst-16-en-3-one (5α-androstenone) is thought to be largely due to microbial action because use of the antibacterial Povidone–iodine markedly reduced the amounts present in collections over 24 h of axillary secretions in men.[7] Further work from these laboratories[8] has clearly identified some *Corynebacterium* spp. as being significantly involved with odour in the axilla and that such strains, whether singly or in combination, can extensively metabolize steroids such as testosterone.[9,10]

Recent studies have revealed that 5,16-androstadien-3β-ol (androstadienol) and 4,16-androstadien-3-one (androstadienone) are present in sterile, adrenaline-induced, apocrine sweat but in small enough quantities that no odour is detectable.[11] Current information strongly supports the hypothesis that the axillary microflora converts androstadienone and androstadienol in apocrine sweat to a mixture of 5α-androstenone, 5α-androst-16-en-3α-ol and 5α-androst-16-en-3β-ol (3α- and 3β-androstenols).[11,12] These steroids have urine- and musk-like odours suggestive of human underarm odour.

The present study describes an investigation to confirm previous findings, to use isolates from the investigation to develop an *in-vitro* model for studying underarm odour in which bacteria metabolize apocrine sweat, and to use the model for identification of specific isolates which are involved in the production of underarm odour.

Methods

Subjects

Thirty-four male subjects, aged 21–55 years were used.

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All were in good health and had had no medication, including antibiotics, for 2 weeks prior to the investigation. They used non-perfumed, non-bacteriocidal soap (Simple Soap, Albion Soap Co. Ltd) and refrained from using antiperspirants and deodorants for at least 5 days before the investigation.

Assessment of underarm odour

The method used was that of Rennie *et al.*,[12] in which a panel of three selected experts was used to assess the odour. On the morning of the test the subjects washed the axillae and were assessed 6 h later by sniffing left and right axillae at a distance of 3 cm from the axillary vault. The odour was recorded on a scale of low, medium or high. There was no discrepancy between the assessors in assigning the class for each axilla or person.

Microbiology

Type strains of bacteria were kindly provided by Professor W.C.Noble, United Medical and Dental Schools of St Thomas's Hospital, University of London. The axillary microflora were sampled using a 2-cm-diameter skin area at the axillary vault.[13] The skin was scrubbed for 30 s with 1·5 ml 0·1 M phosphate buffer pH 7·8 containing 0·1% (v/v) Triton X-100 (BDH). This was repeated and the two samples pooled. Left and right axillae were separately sampled. The samples were decimally serially diluted in half-strength wash fluid and plated on to various recovery media for viable counting.[14] Colony forming units (cfu) and isolates were then identified.[14]

In-vitro model for underarm odour

Two 5 ml distilled diethyl ether washes were taken from two areas of each of the axillary vaults in a similar manner to the buffer wash for bacterial sampling. The extracts were pooled (20 ml) and immediately filtered through sterile 0·22 μm porosity cellulose acetate filters (Oxoid) to remove bacteria. Aliquots (1 ml) of extract were dispensed into 15 ml amber glass bottles with polypropylene caps. The diethyl ether was evaporated with filtered nitrogen and the tubes were sealed and stored at −80°C. Test bacterial isolates were grown at 37°C for 2 days aerobically (anaerobically for propionibacteria) and the cells were harvested by centrifugation at 3000 *g*, washed once with sterile 0·1 M phosphate buffer (pH 6·0) and then resuspended in fresh buffer. The cell suspensions were adjusted to OD_{500} 1·0 with buffer. Cell suspension (1 ml) was added to a bottle of dried axillary extract and the odour assessed after 6 h incubation at 37°C. Odours were scored on a scale of 0–5. Two controls were used, one with no cell suspension and one with no axillary extract.

Statistics

Statistical analysis was performed as recommended by Sokal and Rollf.[15]

Results

Preliminary experiments confirmed that the regime of washing the axillae, followed by odour assessment after 6 h, was an acceptable method. Odour was maximal after 6 h in low-, medium- or high-odour subjects (Fig. 1).

Axillary microflora

The per cent carriage and population densities of the groups of micro-organisms isolated from the axillae are shown in Table 1. There was no qualitative or quantitative difference between the left and right axillary microbial flora. Aerobic coryneforms, propionibacteria, staphylococci and micrococci were the major axillary microbial organisms. All but one person carried detectable aerobic coryneforms (>$\log_{10}$ 0·28 CFU/cm^2). Only two subjects carried Gram-negative bacteria, *Escherichia coli*, at $\log_{10}$ CFU/cm^2 of 2·75 and 4·05. One subject carried appreciable levels of yeasts ($\log_{10}$ CFU/cm^2 of 3·01).

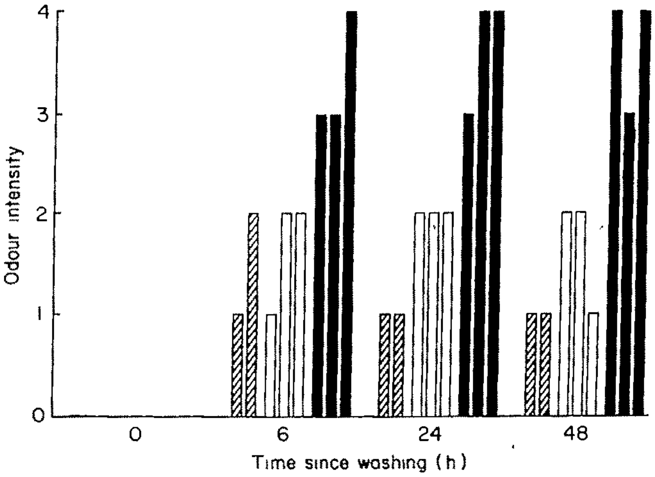

Figure 1. Development of odour intensity in the axillae of three low-, three medium- and three high-odour subjects following washing. ▨, low odour; □, medium odour; ■, high odour.

Table 1. The per cent carriage and population density of microbial groups in the axillae of 34 male subjects

	Mean of both axillae		Left axilla		Right axilla	
	% Carriage	Density†	% Carriage	Density†	% Carriage	Density†
Total bacteria	100	5·85±0·16	100	5·87±0·16	100	5·82±0·16
Aerobic coryneforms	97·1	5·79±0·30	97·1	5·81±0·31	97·1	5·76±0·29
Total *Micrococcaceae*	100	4·76±0·37	100	4·79±0·37	100	4·73±0·37
Staphylococcus	85·3	4·38±0·51	85·3	4·39±0·52	85·3	4·37±0·50
Micrococcus	52·9	1·90±0·52	52·9	1·91±0·52	52·9	1·88±0·52
Propionibacterium	100	3·66±0·35	100	3·63±0·35	100	3·68±0·35
Gram-negative bacteria	5·9	*	5·9	*	5·9	*
Yeasts	2·9	*	2·9	*	2·9	*

*Density not calculated due to low number of carriers.
†Geometric mean log CFU/cm^2±SEM.

Of the 33 isolates of staphylococci, 60% were *S. epidermidis*, 12% *S. haemolyticus*, 6% *S. hominis*, 3% *S. capitis* and 3% *S. saprophyticus*, respectively. *Micrococcus luteus* was the only micrococcus isolated. Fifteen per cent of the isolates could not be identified with confidence to species. Of the 32 propionibacterial isolates, 46·9% were *P. acnes*, 40·6% were *P. avidum* and *P. granulosum* was less prevalent at 12·5%.

Association of the axillary microflora with underarm odour

The geometric mean population densities of the axillary microflora for the three classes of subjects, low, medium and high odour, are shown in Figure 2. There was a strong association between the population density of aerobic coryneforms and the intensity of odour (Spearman, $P=0·001$). An increase of the geometric mean population density of 300 times was associated with a change from low- to high-odour class. Due to the numerical dominance of the aerobic coryneforms in the axillae of high-odour subjects, there was an association of total bacterial microflora and intensity of odour (Spearman, $P=0·001$). No associations were found for intensity of odour and the population densities of the other microbial residents.

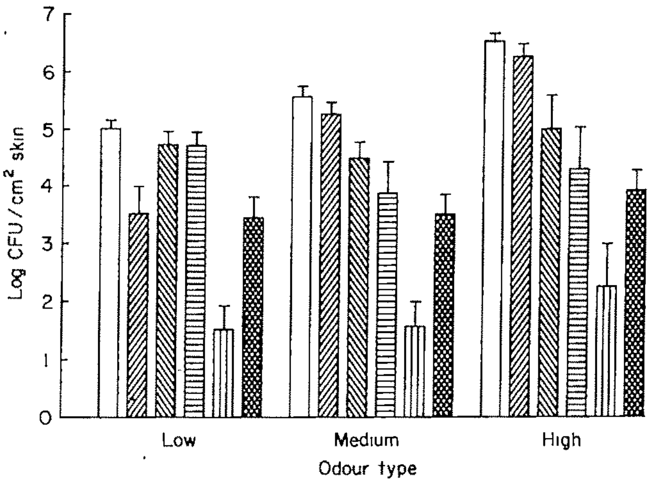

Figure 2. The axillary skin microflora of 10 low-, 10 medium- and 10 high-odour subjects. □, Total microflora; ▨, aerobic cornyneforms; ■, *Micrococcaceae*; ▤, micrococci; ▩, propionibacteria.

Numerical dominance by bacterial groups was an important factor in determining a subject's odour intensity. Where aerobic coryneforms outnumbered *Micrococcoceae*, there was higher odour than when the aerobic coryneforms were not dominant (χ^2, $P=0·005$). There was no evidence of any adverse interaction amongst the bacterial groups (Fisher's exact test, $P>0·05$). Within the sample population there was a slight bias for higher odour in the right axillae. Repeated determinations over 3 days with five subjects revealed remarkable consistency in microbial flora and odour intensity, and where there was a bias of higher odour in the right axillae, this was also consistent.

Development of the in-vitro model for underarm odour

Six isolates of aerobic coryneforms were tested for the production of underarm odour using ether extracts obtained from four low-, five medium- and four high-odour subjects, equivalent to 1 cm^2 skin (Fig. 3). Intense underarm odour was produced by four of the six test bacterial isolates whether the ether extracts were derived from low-, medium- or high-odour subjects. Two positive and two negative odour-producing bacterial

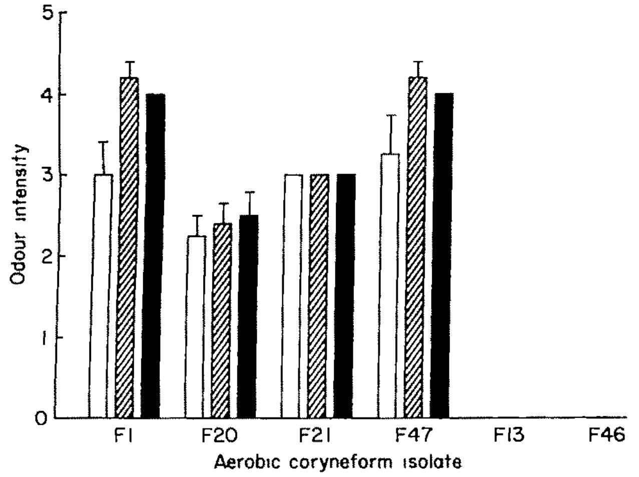

Figure 3. Odour production from extracts of low-, medium- and high-odour producing people by aerobic coryneform isolates. □, low odour extract; ▨ medium odour; ■, high odour.

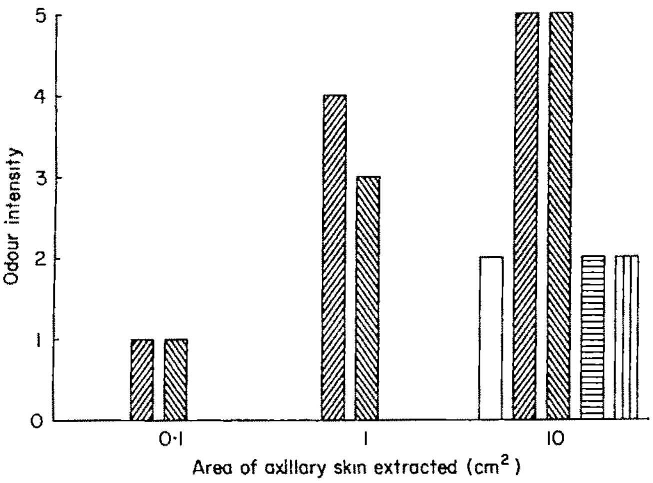

Figure 4. The effect of extract quantity on odour production by aerobic coryneform isolates. □, control; ▨, F1 isolate; ▧, F20, ▩, F13, ▤, F46.

isolates were tested with extracts pooled from three medium-odour subjects at concentrations equivalent to 0·1, 1·0 and 10 cm^2 skin (Fig. 4). An increase in ether extract substrate was accompanied by an increase in intensity of odour. However, at the highest concentration tested, equivalent to 10 cm^2 of skin, the control had a detectable odour and the two negative odour-producing bacterial isolates had the same odour intensity as the control. Therefore, further experiments were undertaken using an extract concentration equivalent to 1 cm^2 skin area, and for consistency pooled extracts were obtained from the same three medium-odour subjects. These pooled extracts were used to determine the effect of pH, cell concentration, physiological state and viability and incubation time on odour production. All incubations were at 37°C with isolate FI, a high-odour producer (Fig. 5). Odour production was optimum at pH 6, and none was detected at pH 4 and 8. Only above cell concentrations of 10^5 CFU/ml was odour production detected which with increasing cell concentration became more intense. Odour production was detected after 1 h incubation and increased up to 5 h but thereafter no increase was detected. Cells harvested from the exponential or stationary phase of growth were equally capable of producing odour. However, the rate of odour production was less with stationary-phase harvested cells.

When cells were heat-killed by boiling for 5 min (less than log 0·30 CFU/ml) and tested at pH 6·0, 37°C for 6 h, no odour could be detected. Viable cells (log 7·82 CFU/ml) gave an odour intensity score of 4.

Finally, the model was tested using extracts obtained from the vault of the axilla, upper back and forearm, from a low-, a medium- and a high-underarm-odour subject. The extracts were used at an equivalent 1 cm^2 skin area and at 37°C for 6 h (pH 6·0) with four positive and two negative odour-producing aerobic coryneforms. No underarm odour was produced from any of the three extracts with the two negative odour-producing bacteria. Underarm odour was produced only from the four positive isolates in the presence of extracts obtained from the axillary vault and fossa. In all cases, the odour intensity was greater with the vault extracts than the fossa extracts.

In-vitro underarm odour production by axillary micro-organisms

The *in-vitro* model, using pooled axillary vault extracts, equivalent to 1 cm^2 skin, pH 6·0 and 6 h incubation at 37°C under aerobic conditions was used to test odour production by a range of bacteria isolated from the axillae (Table 2). Only aerobic coryneforms, and only those isolates identified as *Corynebacterium xerosis*, were capable of producing underarm odour in the *in-vitro* model. Of the *C. xerosis* isolates, 10·5% did not produce odour. The data from the *in-vitro* odour production experiment and the *in-vivo* data on microbial population density of the axillae were combined to examine the *in-vivo* odour intensity and the prevalence and dominance of odour-producing bacteria at the axillary skin site (Table 3). The prevalence of odour-producing bacteria was lowest in low-odour subjects, and these bacteria when present represented a very small proportion of the total microflora. However, in medium- and high-odour

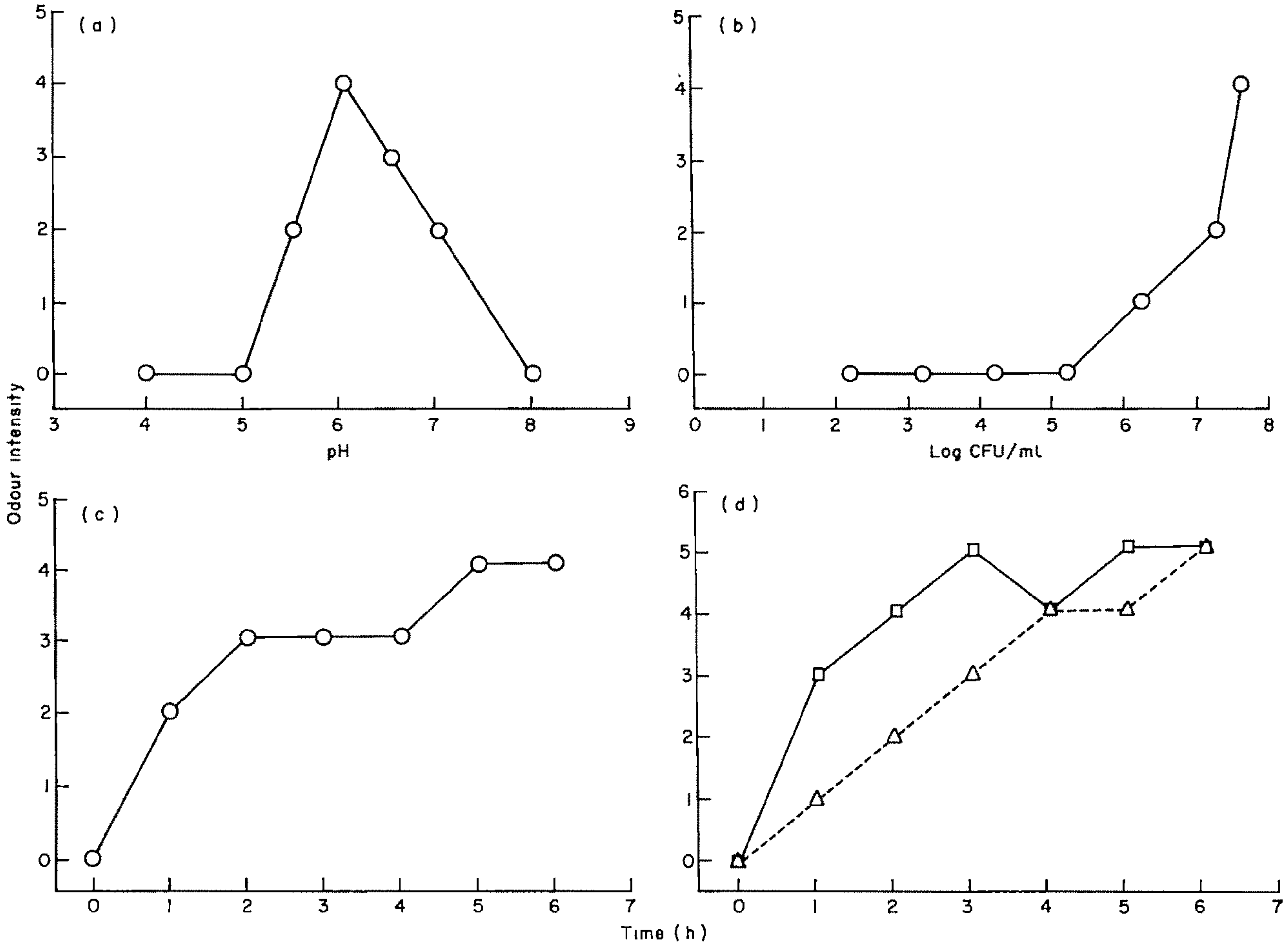

Figure 5. The effect of (a) pH, (b) cell concentration, (c) incubation time and (d) growth phase (□, log phase; △, stationary) on odour production by aerobic coryneform F1.

Table 2. Prevalence of odour formation by bacteria isolated from the axillae tested in the *in-vitro* model.

Bacterium	Number	% Odour producers
Aerobic coryneforms	49	71·4
Corynebacterium xerosis	28	89·5
Others	10	0
Micrococceae	56	0
Propionibacteria	32	0

subjects, only in one individual were there no detectable odour-producing aerobic coryneforms. In medium- and high-odour subjects these bacteria represented a major group of bacterial residents and there were significant increases in the population densities with increasing odour class.

Discussion

This study confirmed previous findings that the axillae in humans support a stable microflora in which the bacterial groups are limited to aerobic coryneforms, members of the *Micrococcaceae* and propionibacteria.[1,16] Although there was variance between individuals in that their microflora, especially the aerobic coryneforms, ranged from none detectable to 10^7 CFU/cm^2 skin, there was high stability in the same person and between left and right axillae. Labows *et al.*, studying 12 males and 12 females, also demonstrated this stability.[2] These investigations suggest that the axillary environmental factors that control the microflora are themselves stable, but differ between individuals. In this study, no evidence could be found for competition between the main bacterial groups, indicating that they inhabit different niches. However, there was a negative association

Table 3. The prevalence, population density and dominance of odour-producing aerobic coryneforms in the axillae of subjects with varying underarm odours.

	Subject odour		
	Low ($n=7$)	Medium ($n=10$)	High ($n=10$)
% prevalence	71·4	90	100
		P† = 0·01	
Geometric mean CFU/cm^2 ± SEM	2·81±0·68	4·79±0·54	6·13±0·13
	P* = 0·04		P* = 0·03
		P* = 0·001	
% dominance of total microflora	0·005	20	39·8

* *t*-test.
† χ^2 test.

between staphylococci and micrococci, suggesting that they compete for the same niche.

Dominance, defined as the highest number of cells, was associated with the density of the total flora. High total flora was associated with aerobic coryneform dominance whilst low total flora was dominated by *Micrococcaceae*. Bacterial population density was associated with dominance with a 75% criterion being used for dominance.[17] The present investigation largely confirmed other studies where a 10% prevalence of *S. aureus* in the axillae was found in one study[1] and 3% reported in another,[18] but none were detected in the present study. The prevalence of Gram-negative bacteria was low (6%), as found by Noble and Somerville,[19] differing from the 20% reported by Leyden *et al.*[1]

The present study showed a significant association between the population density and dominance in the microflora of aerobic coryneforms and underarm odour. Some of the bacteria in this group are important in odour production as was emphasized by Leyden *et al.*[1] Isolates within this group were used to develop a successful *in-vitro* model for underarm odour. Previously reported models using apocrine sweat succeeded in producing underarm odour, but these were restricted because of the very limited amount of apocrine sweat collected.[1,20] The model reported here, using ether extracts from the axillae, required only the equivalent of amounts from 1 cm^2 of skin and was independent of the donor's odour score. Consistency of the results from the model was high for each aerobic coryneform tested. The optimum conditions for underarm odour production were similar in the model to those in the axilla *in vivo*, with odour production reaching a plateau after 6 h incubation at pH 6·0. This pH is close to that reported for axillary skin.[21] Underarm odour could be produced by cells in the exponential and stationary phase of growth but not by heat-killed cells. This suggests that although viable cells are capable of odour production, non-viable but metabolically active cells may also cause odour production. Whatever the cell state, the odour is dependent on the number of cells present.

When the *in-vitro* model was used to test odour production by a wide range of axillary bacterial isolates, only those in the aerobic coryneform group were positive, confirming their crucial role in underarm odour. Equally important was the finding that not all aerobic coryneforms were odour producers. This may explain why carriage of aerobic coryneforms may not be sufficient to cause odour. For example, nearly 30% of low-odour subjects did not carry odour-producing aerobic coryneforms, but if they did, then they were present in trivial numbers compared to the rest of the microflora. In this context, it is significant that the *Corynebacterium* spp. which produced odour in the *in-vivo* test described here, can metabolize steroids such as testosterone and 16-androstenes. Underarm-odour-negative strains were found to be largely incapable of carrying out such transformations, as were *Propionibacterium* spp.[22]

The present investigation has quantitatively and qualitatively defined the axillary microflora that is specifically involved in underarm odour production. Although underarm odour cannot be described in terms of specific skin substrate(s) used, enzymes involved and odoriferous compounds produced, the *in-vitro* model has opened up possibilities for future investigations of these topics.

References

1 Leyden JJ, McGinley KJ, Hobie E *et al.* The microbiology of human axilla and its relationship to axillary odour. *J Invest Dermatol* 1981, 77: 413–6.

2 Labows JN, McGinley KJ, Kligman AM. Perspectives on axillary odour. *J Soc Cosmet Chem* 1982; 34: 193–202.

3 Shehadeh N, Kligman AM. The effect of topical antibacterial agents on the bacterial flora of the axilla. *J Invest Dermatol* 1963; 40: 61–71.

4 Baxter PM, Reed JV. Evaluation of deodorants. *Proc Soc Cosmet Chem Symp* Substantiation of Cosmetic Benefits. Eastbourne, Sussex; 1982.

5 Preti G, Cutler WB, Christensen CM *et al.* Human axillary extracts: Analysis of compounds from samples which influence menstrual timing. *J Chem Ecol* 1987; 13: 717–731.

6 Nixon A, Mallet AI, Gower DB. Simultaneous quantification of five odorous steroids (16-androstene) in the axillary hair of men. *J Steroid Biochem* 1988; **29**: 505–510.

7 Bird S, Gower DB. Axillary 5α-androst-16-en-3-one, cholesterol and squalene in men: preliminary evidence for 5α-androst-16-en-3-one being a product of bacterial origin. *J Steroid Biochem* 1982; 17: 517–522.

8 Rennie PJ, Holland KT, Mallet AI *et al.* The skin microflora and the formation of human axillary odour. *Int J Cosmet Science* 1990; 12: 197–207.

9 Nixon A, Mallet AI, Jackman PJH, Gower DB. Testosterone metabolism by isolated human axillary *Coryneform* spp: a gas chromatographic–mass spectrometric study. *J Steroid Biochem* 1986; **24**: 887–893.

10 Nixon A, Jackman PJH, Mallet AI, Gower DB. Testosterone metabolism by pure and mixed culture of human corynebacteria. *FEM Microbiol Lett* 1987; **41**: 53–58.

11 Rennie PJ, Holland KT, Mallet AI *et al.* Interconversion of androst-16-ene steroids by human axillary aerobic coryneform bacteria. *Biochem Soc Trans* 1989; **17**: 1027–8.

12 Rennie PJ, Holland KT, Mallet AI *et al.* 16-Androstene content of apocrine sweat and microbiology of the human axilla. In: *Chemical Signals in Vertebrates V.* (McDonald D, ed). Oxford: Oxford University Press, 1990; 55–60.

13 Williamson P, Kligman AM. A new method for the quantitative investigation of cutaneous bacteria. *J Invest Dermatol* 1965; **45**: 498–503.

14 Marshall J, Leeming JP, Holland KT. The cutaneous microbiology of normal human feet. *J Appl Bacteriol* 1987; **62**: 139–46.

15 Sokal RR, Rolf FJ, Biometry. 2nd edn. San Francisco: W.H. Freeman.

16 Prince H, Rodgers JA. Studies in the aerobic axillary microflora employing a standardised swabbing technique. *Cosmet Perfum* October 1974.

17 Jackman PJ, Noble WC. Normal axillary skin microflora in various populations. *Clin Exp Dermatol* 1983; **8**: 259–68.

18 Somerville DA. The normal flora of the skin in different age groups. *PhD Thesis*, University of Otago, New Zealand. 1966.

19 Noble WC, Somerville DA. *Microbiology of Human Skin.* London: W.B.Saunders, 1981.

20 Hurley HJ, Shelley WB. *The Human Apocrine Sweat Gland in Health and Disease.* Springfield: C.C.Thomas, 1960.

21 Behrendt H, Green M. *Patterns of Skin pH from Birth Through Adolescence.* Springfield: C.C.Thomas, 1971.

22 Mallet AJ, Holland KT, Rennie PJ *et al.* Applications of gas chromatography–mass spectrometry in this study of androgen and odorous 16-androstene metabolism by human axillary bacteria. *J Chromatog* 1990; **562**: 647–58.

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Acantholytic dyskeratotic epidermal naevus in a patient with guttate psoriasis on PUVA therapy

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Summary A 79-year-old male with psoriasis developed a zosteriform acantholytic dyskeratotic epidermal naevus during PUVA therapy. The eruption was successfully treated with etretinate 0·75 mg/kg/day and a subsequent course of PUVA with the affected area covered prevented a recurrence of the naevus.

Classical keratosis follicularis (Darier's disease) is a well-recognized entity with acantholysis, dyskeratosis and the presence of corps-ronds. These histological features, however, are not pathognomonic for Darier's disease[1] and may also be present in other disorders such as transient acantholytic dermatosis or acantholytic dyskeratotic epidermal naevus. The latter condition was originally described as a 'forme fruste' of Darier's disease.[2] The naevoid nature of the eruption is however more likely[3,4] and is characterized as an acantholytic dyskeratotic epidermal naevus on the basis of its delayed onset, the absence of a family history and that the condition is provoked by hot and humid weather.[5] Its onset following sun exposure has also been described.[6,7] We present a case of acantholytic dyskeratotic epidermal naevus that occurred during PUVA treatment in a 79-year-old patient with psoriasis.

Case report

A 79-year-old Caucasian male developed a non-itching linear papular eruption on the right side of his chest during the third month of PUVA treatment for severe guttate psoriasis. The total dose of UVA that he had received at the time was 40 J/cm^2. The lesions tended to follow Blaschko's lines[8] and despite protection of the affected area from the light the eruption extended during the next 2 weeks of PUVA therapy. There was no family history of Darier's disease.

The patient had lenticular crusted and keratotic

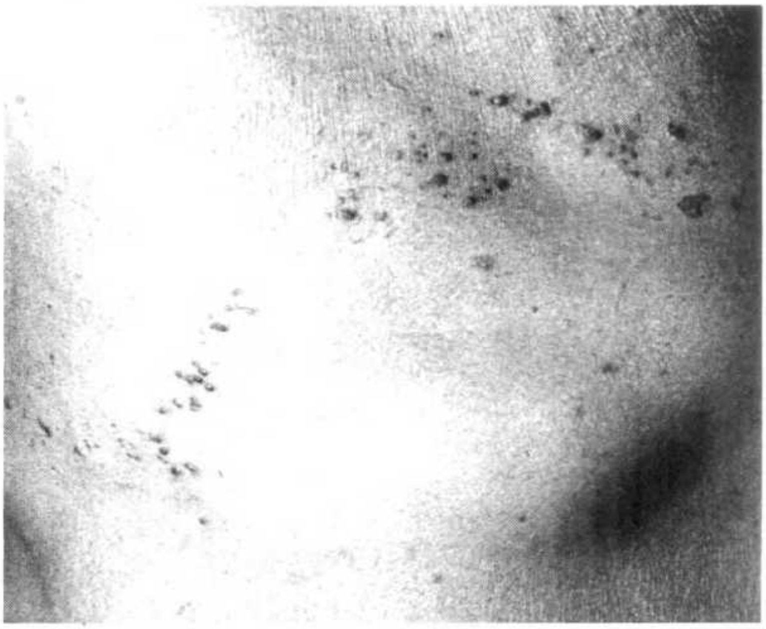

Figure 1. Linear distribution of papules on the right side of the thorax.

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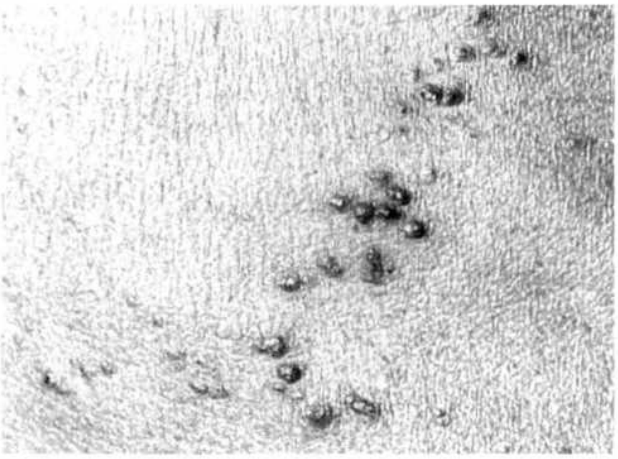

Figure 2. Crusted hyperkeratotic papules.

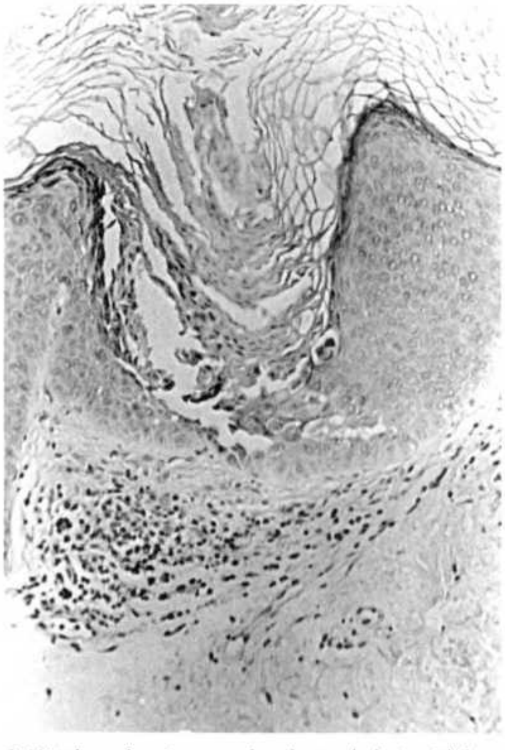

Figure 3. Histology showing suprabasal acantholysis with hyperkeratosis, focal parakeratosis and dyskeratosis (× 20).

papular lesions on the right side of the chest (Figs 1 and 2). There were no nail changes, palmar pits or mucosal lesions and only the non-psoriatic skin was affected. The clinical diagnosis of acantholytic dyskeratotic epidermal naevus was made and a skin biopsy showed hyperkeratosis with focal parakeratosis, dyskeratosis and suprabasal acantholysis of the epidermis (Fig. 3). Corps-ronds were occasionally found in the granular layer. The dermis showed a mainly lymphocytic infiltrate.

Treatment with PUVA was discontinued as it was regarded as a possible provoking factor for the acantholytic dyskeratotic epidermal naevus which was treated topically with 0·05% retinoic acid in a cetomacrogol cream. This appeared to exacerbate the guttate psoriasis and therapy was then changed to etretinate (Tigason®) 0·75 mg/kg/day. Both his psoriasis and the naevoid condition resolved almost completely within 2 months. PUVA therapy was again given because the side-effects from etretinate meant that it had to be discontinued. The area previously involved with the acantholytic dyskeratotic epidermal naevus was covered during treatments, and 1 month later the psoriasis had cleared without any recurrence of the naevus. No provocation tests were done with PUVA because shortly afterwards the patient died from an unrelated cause.

Discussion

Acantholytic dyskeratotic epidermal naevus is an uncommon disorder with a delayed onset, the lesions starting from the age of 20 onwards. The occurrence of the condition in elderly patients has been described. Most of the patients improve with topical applications of vitamin A acid,[6] but in our patient this treatment was ineffective, although systemic therapy with etretinate cleared the lesions.

A patient was described in whom acantholytic dyskeratotic epidemal naevus-like lesions were diagnosed which later could be provoked by UVB radiation.[6] Irradiation with UVA without psoralens did not induce the lesions. Our patient developed an acantholytic dyskeratotic epidermal naevus during his first course of PUVA treatment. Although he had previously had abundant exposure to the sun, he had never had evidence of this naevus. We believe that the occurrence of this was provoked by his PUVA treatment and that photoprotection of the area of skin previously affected was sufficient to prevent a recurrence during the second course.

References

1 Ackermann AB. Focal acantholytic dyskeratosis. *Arch Dermatol* 1972; **106**: 702–6.
2 Anderson NP. Darier's disease (unilateral). *Arch Dermatol Syphilol* 1948; **58**: 581–3.
3 Leeming JAL. Acquired linear naevus showing histological features of keratosis follicularis. *Br J Dermatol* 1969; **81**: 128–31.
4 Demetree JW, Lang PG, St Clair JT. Unilateral, linear, zosteriform

epidermal naevus with acantholytic dyskeratosis. *Arch Dermatol* 1979; 115: 875–919.

5 Starink ThM, Woerdeman MJ. Unilateral systematised keratosis follicularis. A variant of Darier's disease or an epidermal naevus (acantaolytic epidermal naevus)? *Br J Dermatol* 1981, 105: 207–14.

6 Baba T, Yaoita H. UV radiation and keratosis follicularis. *Arch Dermatol* 1984; 120: 1484–7.

7 Thomas I, Shockman J, Epstein JD. Linear keratosis follicularis: a specific entity? *J Am Acad Dermatol* 1989; 20: 1122–3.

8 Happle R, Frosch PJ. Manifestation of the lines of Blaschko in a woman heterozygous for X-linked hypohidrotic ectodermal dysplasia. *Clin Genet* 1985; 27: 469–71

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Erythroderma, palmoplantar keratoderma and profound failure to thrive in an infant

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Summary The case is reported of a female infant, who at the age of 3 months developed severe erythroderma, marked hyperkeratosis of the palms and soles and subsequently extreme growth failure and intermittent diarrhoea. Her course was complicated by life-threatening infections but detailed investigation revealed no recognized underlying metabolic or immune abnormality.

Case report

A 2·7-kg female infant, born at 36 weeks gestation after a normal pregnancy, was noted at birth to have dry, red, tight skin with no evidence of blistering, infection or collodion membrane. The skin improved with the application of emollients and was reported to be normal at 2 weeks of age. She fed well and gained weight.

At the age of 3 months she developed a blotchy red, scaly rash on the face, scalp and nappy area which slowly spread to the body and limbs, becoming confluent within 2 weeks. She was admitted to hospital and the eruption partially regressed on treatment with topical emollients and steroids in addition to oral antibiotics. Rapid relapse led to readmission 1 week later and in addition to a generalized scaly erythroderma she had bronchiolitis necessitating intravenous antibiotics and oxygen therapy.

At the age of 4 months, she was referred to Great Ormond Street Hospital with a severe generalized ichthyosiform erythroderma, diffuse skin thickening and palmoplantar hyperkeratosis. There were fissures in the flexural areas, thick waxy scales on the scalp and legs and fine white scales elsewhere, and in addition she had total alopecia and dystrophy of all her nails (Fig. 1). She was alert but irritable and, at 4·3 kg and 56 cm, well below the 3rd percentiles for weight and height.

There was no family history of any similar skin or genetic disorder.

Investigations

Initial investigations showed a leucocytosis of $22 \cdot 7 \times 10^9$ with a normal haemoglobin and platelet count and an ESR of 2 mm/h. Clotting studies, platelet function tests, serum and RBC folate and vitamin B_{12}, corrected calcium, phosphate, magnesium, copper, zinc and selenium levels were all normal. Total protein at 35 g/l and albumen at 18 g/l were markedly reduced. Urea, creatinine, electrolytes, liver function tests, immunoglobulins and TSH were normal. A full autoantibody and complement profile was negative. T-cell subset numbers and function were within normal limits. There was a minimal reduction in C3b uptake which was not thought to be significant.

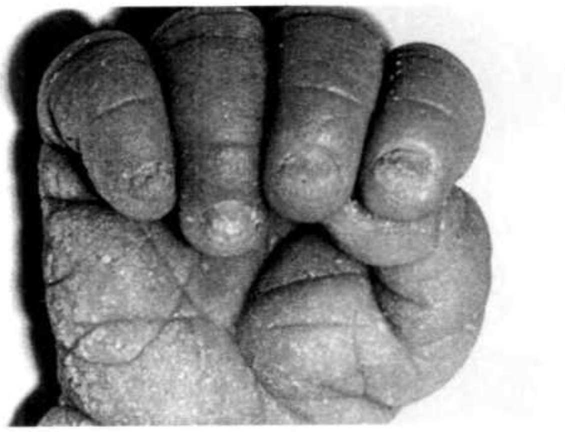

Figure 1. Nail dystrophy and palmar keratoderma.

Radiological studies, including chest X-ray and ultrasound of the abdomen, detected no abnormalities.

Histology

A skin biopsy from the upper back revealed parakerato-

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sis, mild acanthosis and a chronic inflammatory cell infiltrate in the upper dermis.

A jejunal biopsy was reported as showing normal villous and crypt architecture, with no increase in intraepithelial lymphocytes and no evidence of lumenal micro-organisms.

Progress and management

One week after admission she was febrile and unwell. Blood cultures grew Gram-positive cocci and she responded to appropriate intravenous antibiotics although she continued to have febrile illnesses necessitating repeated courses of antibiotics. Persistent tachypnoea occurred, in the absence of clinical or radiological signs. Hepatomegaly, with normal liver function tests, was also noted.

In spite of an oral milk and solid food intake of 165 kcals/kg/24 h (recommended intake for a healthy infant at this age: 100–130 kcals/kg/day) there was continuing weight loss to 3·6 kg, persistent hypoalbuminaemia (12 g/l), oedema and intermittent loose stools. There was no evidence of protein loss through enteropathy or nephropathy. She was given several infusions of albumin but this did not improve the serum albumin levels or clinical state. Intravenous feeding was commenced through a central long line and continued for a total of 5 months, during which time she slowly gained weight (Fig. 2).

During this period she had several episodes of septicaemia due to a variety of organisms including *Staphylococcus aureus* and *S. epidermidis*, *Streptococcus faecalis*, *Pseudomonas aeruginosa* and *Candida albicans*. Virtually continuous intravenous antibiotic and antifungal therapy was required and alternate weekly immunoglobulin and fresh frozen plasma infusions were given over a period of 6 months to augment yeast opsonization. The central line had been resited three times but, because of recurrent infection, was finally removed. Intravenous feeding was continued for 3 weeks through a peripheral line and it too became infected with Staphylococcal abscesses at the insertion site.

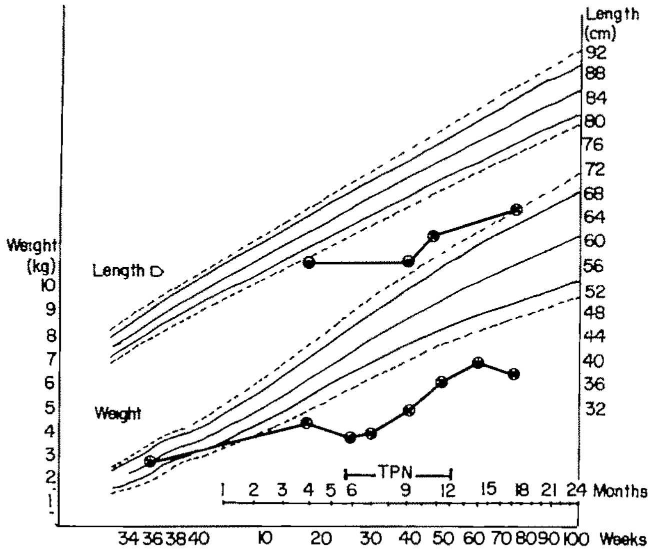

Figure 2. Growth chart. Note weight gain while on total parenteral nutrition (TPN).

At the age of 11 months her weight had risen to 6·2 kg. Enteral feeding via a nasogastric tube was gradually increased (she showed no interest in oral feeding) and, apart from frequent semi-formed motions, this was well tolerated. Her weight slowly climbed to 6·5 kg with an associated growth spurt to 61 cm, both parameters still below the third percentiles.

She was treated with frequent oily baths and moisturizing creams but the erythroderma persisted with increasing pruritus and the palmoplantar keratoderma became more pronounced. Other treatments that were tried, without success, included 1% topical hydrocortisone, topical linoleic acid and a course of parenteral and oral indomethacin in an effort to modify the inflammatory process.

Further pyrexial episodes occurred, even after the indwelling line had been removed, and marked hepatosplenomegaly was now evident though her overall clinical state had improved. Full immune studies were normal at this stage and intravenous immunoglobulins were discontinued.

She was finally discharged to the care of her local hospital at the age of 14 months when established on a combined nasogastric and oral intake equivalent to 190 kcals/kg/day. Her weight had reached 7 kg. Pyrexial episodes continued but blood cultures were negative. Her weight fell at 18 months and overnight nasogastric feeding was commenced. At the age of 20 months she suffered a severe chest infection complicated by congestive cardiac failure and following this episode remained oxygen dependent.

Increasing keratoderma around the knees was evident, nail dystrophy persisted and there was minimal fluffy scalp hair growth. During intervals at home her motor and social skills slowly improved but she achieved only the 8–9 month level of development when 20 months of age.

Discussion

This infant developed a severe generalized erythroderma, failure to thrive, palmoplantar keratoderma, nail dystrophy and hypotrichosis at the age of 3 months. In addition she had marked hypoalbuminaemia, in spite of high protein intake, and recurrent life-threatening infections in the face of normal immune function. She remained critically ill for 9 months and thereafter showed signs of a modest clinical improvement though the erythroderma and growth failure persisted.

There are a number of disorders that may present with erythroderma in infancy including seborrhoeic dermatitis, atopic eczema, congenital psoriasis, the ichthyosiform erythrodermas, Netherton's syndrome, immunodeficiency syndromes such as Ommen's syndrome, diffuse mastocytosis and multiple decarboxylase deficiency.

Generally, it is possible to identify major immunodeficiency disorders, mastocytosis and biotin deficiency with the appropriate investigations but the other disorders may be difficult to distinguish until later in the course of the disease when additional characteristic features become manifest. In many such children the presentation of erythroderma with failure to thrive is considered a poor prognostic sign and in the past these were included in the diagnostic category of Leiner's syndrome.

Leiner[1,2] in 1908 reported details of 43 infants, of whom 14 died, who had presented with these features within the first month of life. Several similar reports followed and various, often minor, defects in immune studies such as yeast opsonization, complement deficiencies, elevated IgE and abnormal neutrophil motility were identified and thought to account for the high rate of infections seen in these infants.[3–5] It is now recognized that 'Leiner's' represents a heterogeneous group of disorders and it has been proposed that the term no longer be used.[6]

There is no consistent pattern of immune dysfunction in these infants and indeed minor deviations from normal childhood values are common in otherwise healthy young infants. In particular, a yeast opsonization defect occurs in up to 20% of the normal population.[7] Raised IgE levels may be a marker of coexistent atopy or a feature of Netherton's syndrome.

Failure to thrive is likely to be multifactorial, due to poor intake, protein and water loss through the inflamed skin, debility induced by pruritus and pain and intermittent diarrhoea. It is presumed that the inflammatory process may affect the intestinal mucosa as well as the skin, although this is often difficult to demonstrate because histological changes are likely to be patchy and submucosal.

Palmoplantar keratoderma is a rare condition in childhood and of the possible hereditary non-mutilating keratodermas, Unna–Thost and Papillon–Lefevre syndromes are localized conditions[8] and epidermolytic hyperkeratosis[9] shows characteristic histological changes. Tyrosinaemia type 2[10] leads to a late-onset focal, painful keratoderma with corneal damage. Of the other possible causes, pityriasis rubra pilaris was considered but it has not been reported to present in this manner. The association of erythroderma and keratoderma is a feature of certain of the ichthyosiform erythrodermas but otherwise is an unusual combination in infancy and childhood.

In conclusion, we report a child who presented with a new syndrome of unknown aetiology but of presumed genetic causation, either due to a new mutation or to an autosomal recessive trait.

Acknowledgments

We wish to thank Dr Peter Milla and Dr Frank Allenby for their advice and we gratefully acknowledge the care provided by the Sisters and nursing staff of Cohen Ward, The Hospitals for Sick Children, Great Ormond St and the Paediatric Unit, Lister Hospital, Stevenage.

Addendum in proof

Sadly on 22 December 1990, at the age of 22 months, she died as a result of overwhelming infection.

References

1 Leiner C. Über Erythrodermia desquamativa, eine eigenartige universelle Dermatose der Brustkinder. *Arch Dermatol Syphil* 1908; 89: 163–89.

2 Leiner C. Erythroderma desquamativa (universal dermatitis of children at the breast). *Br J Dis Child* 1908, 5: 244–51.

3 Jacobs JC, Miller ME. Fatal familial Leiner's disease: A deficiency of the opsonic activity of serum. *Pediatrics* 1972; 49: 225–32.

4 Scott H, Moynahan EJ, Risdon RA *et al.* Familial opsonization defect associated with fatal infantile dermatitis, infections and histiocytosis. *Arch Dis Child* 1975; 50: 311–17.

5 Evans DIK, Holzel A, McFarlane H. Yeast opsonization defect and immunoglobulin deficiency in severe infantile dermatitis (Leiner's disease). *Arch Dis Child* 1977; 52: 691–5.

6 Glover MT, Atherton DJ, Levinsky RJ Syndrome of erythroderma, failure to thrive and diarrhoea in infancy: A manifestation of immunodeficiency. *Pediatrics* 1988; 81: 66–72.

7 Soothill JF, Harvey BAM. Defective opsonization. a common immunity deficiency. *Arch Dis Child* 1976; 51: 91–9.

8 Griffiths WAD, Marks R, Burton JL. Disorders of keratinization. In: *Textbook of Dermatology* (Rook A, Wilkinson DS, Ebling FJG, Champion RH, Burton JL, eds), 4th edn. Oxford: Blackwell Scientific Publications, 1986; 1452.

9 Ackerman AB. Histopathologic concept of epidermolytic hyperkeratosis. *Arch Dermatol* 1970; 102: 253–9.

10 Goldsmith LA. Tyrosine induced skin disease. *Br J Dermatol* 1978; 98: 119–23.

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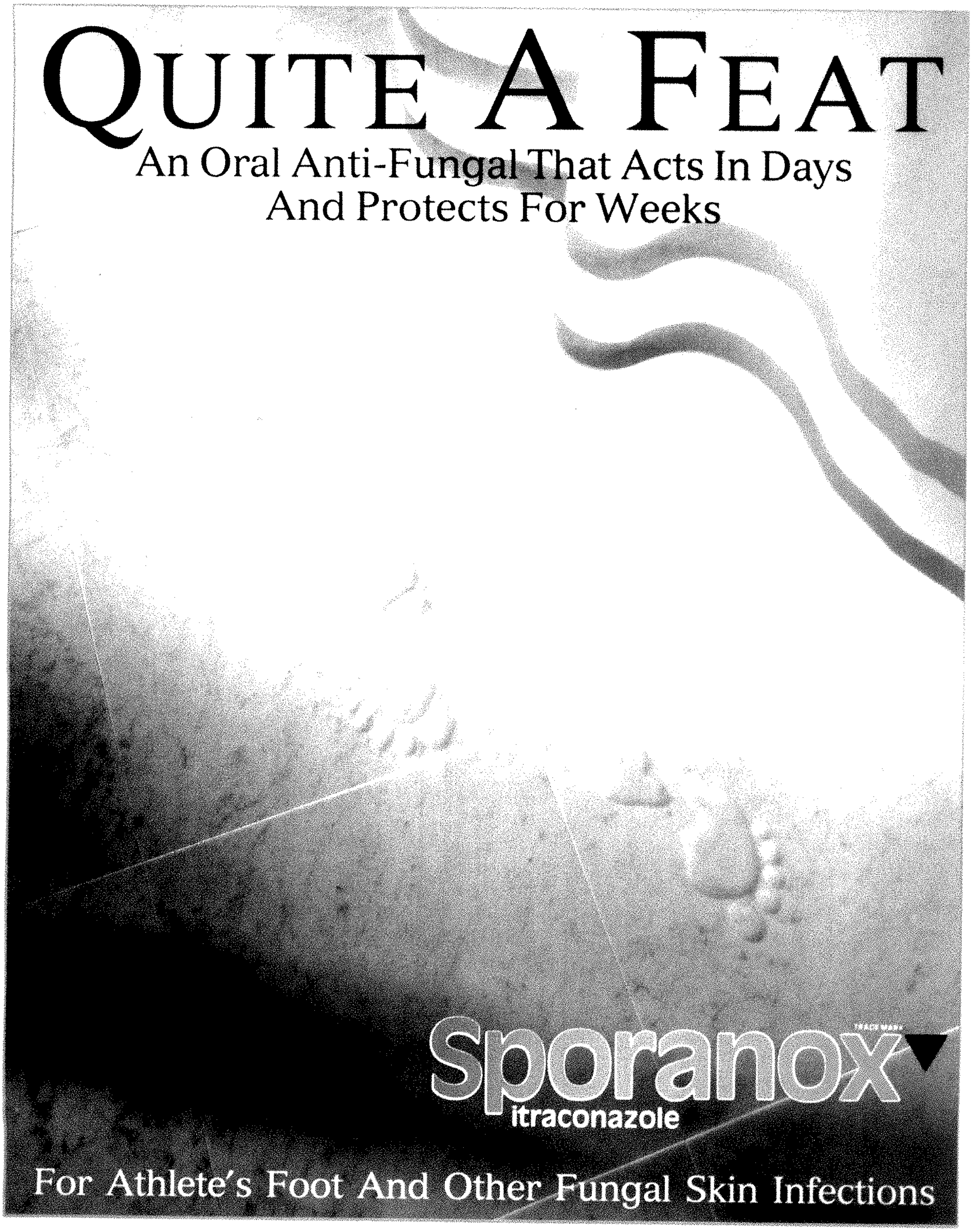
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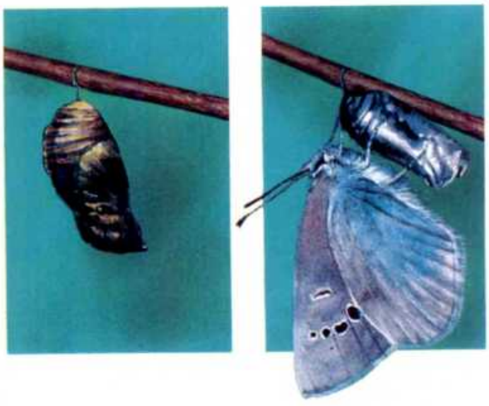

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Correspondence

Pemphigus vegetans presenting with deafness, otalgia and facial nerve paralysis

SIR, We report a patient with pemphigus vegetans of the Hallopeau type who presented with deafness, otalgia and a facial palsy. To our knowledge, this has not been previously reported.[1]

A 63-year-old man noticed in March 1989 gingival bleeding on brushing his teeth and shortly afterwards developed otalgia and deafness. He developed gradual hypertrophy of the gingivae (Fig. 1) and a biopsy taken showed prominent papillomatosis, acanthosis and a dense infiltrate of eosinophils and neutrophils in the dermis and eosinophilic microabscesses in the epidermis (Fig. 2). Direct immunofluorescence showed intercellular IgG in the epidermis and circulating antibodies were detected to intercellular material between epidermal cells on indirect immunofluorescence. Otological examination showed that both auditory canals were obliterated by a papillomatous hypertrophy of their walls and a purulent otorrhoea. There was a marked conduction deafness as shown on audiometry. The patient then developed a left facial nerve palsy. Computerized tomography showed low density material in both external auditory canals and in the middle ear (Fig. 3). A biopsy taken from the left auditory canal showed elongation of the epidermal rete ridges and an inflammatory cell infiltrate in the dermis that was composed mainly of neutrophils.

The patient was treated with oral prednisolone in a dosage of 30 mg/day and there was a dramatic improvement, with the disappearance of the otalgia and improvement of the deafness and facial palsy. His gingival lesions healed almost completely within 2 weeks.

Although there is some controversy about the nosology we diagnosed our case as being that of pemphigus vegetans of the Hallopeau type.[2,3] It is however difficult to differentiate this from pyostomatitis vegetans[4] when the lesions are restricted to the oral mucosa. Intercellular deposits of IgG or IgA have been

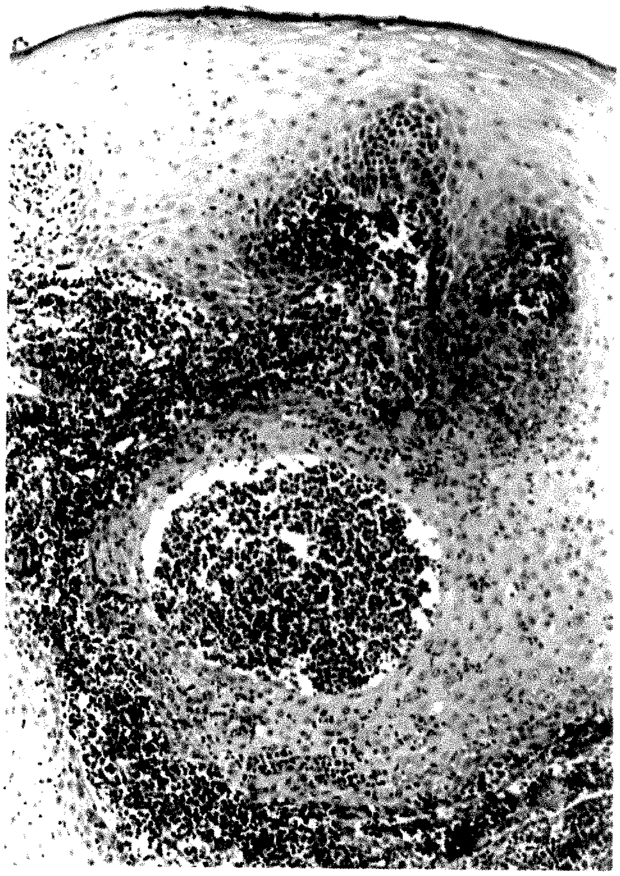

Figure 2. Biopsy from the lower gingiva showing eosinophilic microabscess in the epidermal strand (Haematoxylin and eosin, × 112).

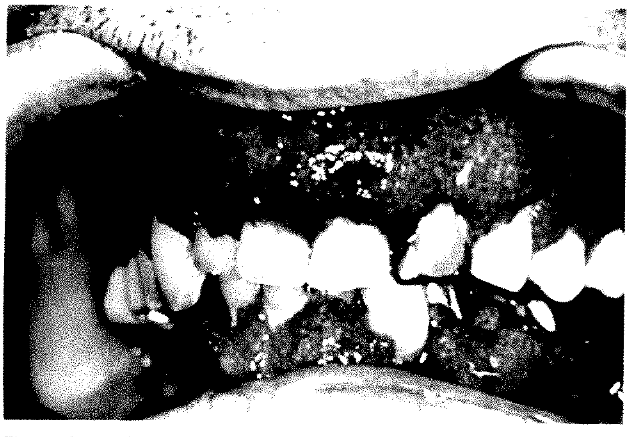

Figure 1. Bright red, granular, hyperplastic gingivae.

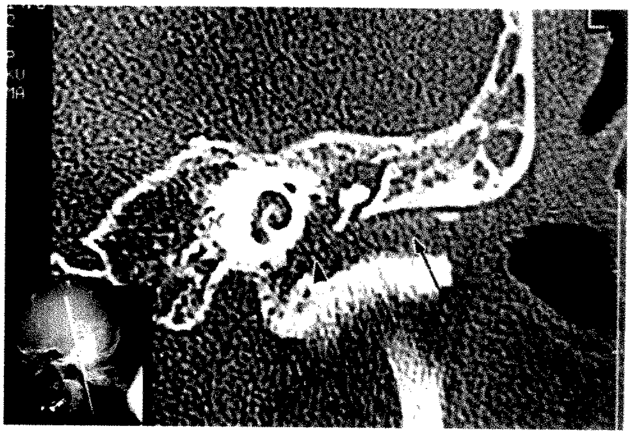

Figure 3. Computarized tomography showing low density material in the left external auditory canal (arrow) and middle ear (arrow head).

detected in some cases of pyostomatitis vegetans,[5] but this diagnosis should be supported by the presence of inflammatory bowel disease that is not seen in pemphigus vegetans.[4-6] The sites of predilection for pemphigus vegetans are the mucous membranes of the mouth and nose and lesions occur in the axillae, groins, genitalia, on the extremities and scalp. In our case vegetating and exudative lesions occurred in both external auditory canals and in the middle ear and were the cause of otalgia, tinnitus, otorrhoea and deafness. The facial palsy that developed may have been due to pressure on the facial nerve either from the inflammatory oedema or resulting from the vegetating lesions in the ear.[7]

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References

1 Ahmed AR, Blose DA. Pemphigus vegetans: Neumann type and Hallopeau type. *Int J Dermatol* 1984; **23**: 135–41.
2 Nelson CG, Apisarnthanarax P, Bean SF, Mullins JF. Pemphigus vegetans of Hallopeau: immunofluorescent studies. *Arch Dermatol* 1977; **113**: 942–5.
3 Neuman HAM, Faber WR. Pyodermite végétante of Hallopeau: Immunofluorescence studies performed in an early disease stage. *Arch Dermatol* 1980; **116**: 1169–71.
4 McCarthy P, Shklar G. A syndrome of pyostomtitis vegetans and ulcerative colitis. *Arch Dermatol* 1963; **88**: 913–19.
5 Ballo FS, Camisa C, Allen CM. Pyostomatitis vegetans: Report of a case and review of the literature. *J Am Acad Dermatol* 1989; **21**: 381–7.
6 Neville BW, Smith SE, Maize JC *et al.* Pyostomatitis vegetans. *Am J Dermatopathol* 1985; **7**: 69–77.
7 Shambaugh GE Jr, May M. Facial nerve paralysis. In: *Otolaryngology* (Paparella MM, Shumrick DA eds), 2nd edn. Philadelphia: W.B.Saunders company, 1980; 1680–1704.

ADONIS 000709639100148S

Malignant melanoma occurring on a 'naevus on naevus'

Sir, The occurrence of malignant melanoma in giant congenital naevi is well documented, although there is some controversy as to its frequency.[1,2] Malignant melanoma on a 'naevus on naevus' however, is rarely reported. We describe a case of superficial spreading melanoma that arose in a large speckled naevus.

A 59-year-old Caucasian woman had from birth a giant pigmented lesion that extended from the right axilla to the pubic region (Fig. 1). It consisted of a large pale-brown cafe-au-lait macule that was speckled by numerous dark-brown maculo-papular lesions. In February 1988, one of these speckled papules in the left groin increased in size, becoming darker and suggestive of a malignant change. This lesion was excised and the histology was that of a superficial spreading melanoma with a nodule whose Breslow thickness was 1·10 mm. At the periphery of the tumour a naevocytic naevus was noted and the adjacent cafe-au-lait macule showed only hyperpigmentation of the basal layer of the epidermis. There was no family history of atypical naevi or of malignant melanoma.

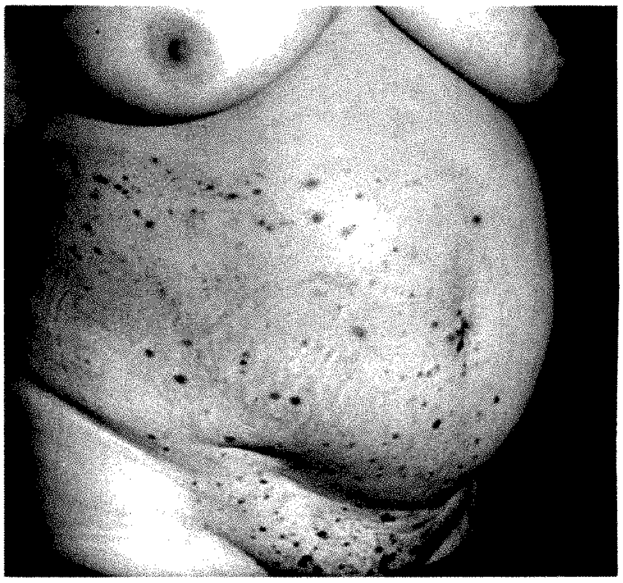

Figure 1. Giant pigmented speckled naevus on the right side of the patient.

This type of speckled 'naevus on naevus', is not usually thought to be associated with malignant change, although since 1985 five cases of malignant melanoma have been reported.[3-7] The mean age of the patients was 59 years at the time of diagnosis of malignant melanoma. The 'naevus on naevus' was congenital in three of the cases,[3,5,7] as in our patient. One melanoma occurred in a large zosteriform naevus.[3] In three of the cases, as in our patient, the melanoma was of the superficial spreading type and was unspecified in two of the cases. In one case the melanoma led to the death of the patient.[5] All the cases of malignant melanoma occurred in the dark-brown speckles with the histological pattern of a naevocytic naevus. We consider that a giant 'naevus on naevus' should be watched, as the condition cannot be regarded as always being benign, and there is a case for the prophylactic excision of a large 'naevus on naevus', as for a giant congenital naevus.

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References

1 Lorentzen M, Pers M, Bretteville Jensen G. The incidence of malignant transformation in giant pigmented naevus. *Scand J Plast Reconst Surg* 1987; 11: 163–7.
2 Reed WB, Becker SW, Becker SW Jr, Nickel WM. Giant pigmented naevi melanoma and leptomeningeal melanocytosis. *Arch Dermatol* 1965; 91: 101–19.
3 Vion B, Belaich S, Grossin M, Préaux J. L'aspect évolutif du naevus sur naevus: Revue de la littérature à propos de 7 observations. *Ann Dermatol Vénéréol* 1985; 112: 813–19.
4 Kopf AW, Levine LJ, Rigel DS *et al*. Congenital naevus like naevi, nevi spili and cafe au lait spots in patient with malignant melanoma. *J Dermatol Surg Oncol* 1985; 11: 275–80.
5 Brufau C, Moran M, Armijo M. Naevus sur naevus: A propos de 7 observations, 3 associées à d'autres dysplasies, et une à un mélanome malin évolutif. *Ann Dermatol Vénéréol* 1986; 113: 409–18.
6 Rhodes A. Neoplasia: benign neoplasia, hyperplasia, and dysplasias of melanocytes. In: *Dermatology in General Medicine* (Fitzpatrich TB, Eisen AZ, Wolff K, Freedberg IM, Austin KF, eds), 3rd edn. New York: McGraw Hill, 1987; 877–945.
7 Wagner RF, Cottel WI. *In situ* malignant melanoma arising in a speckled lentiginous naevus. *J Am Acad Dermatol* 1989; 20: 125–6.

ADONIS 000709639100149I

Cutaneous malignant melanoma occurring after cyclosporin A therapy

SIR, We read with interest the paper by Mérot *et al.* in which they reported the development of cutaneous melanoma in two patients treated with cyclosporin A (CyA) for renal transplantation and systemic sclerosis, respectively.[1] However, we think that some aspects of this paper need to be commented upon.

Malignant melanoma (MM) as such is not an infrequent disease; during 1986 in the U.S.A. 23,000 new cases were diagnosed and 5600 patients died from this cause. Among patients over 65 years of age, the annual incidence rate (1979–81) in the U.S.A. was 26·9 cases per 100,000 inhabitants.[2] Malignant melanomas have been described during therapy with immunosuppressive agents[3–6] and CyA.[7]

The overall incidence of MM in patients treated with CyA is not fully established. In the post-marketing surveillance study of CyA in transplantation, which has followed-up almost 6000 patients, some for up to 7 years, we have not detected any cases of MM. In addition, more than 4000 patients have been treated with CyA for autoimmune diseases and, apart from the first patient mentioned in the paper by Mérot *et al.*,[1] we are only aware of one case in a patient with rheumatoid arthritis, whose lesion was detected 4 months after discontinuation of CyA. This does not seem to be much different from observations in the normal population (see above). Regarding the first case described by Mérot *et al.*,[1] in their paper they did not mention the fact that after this melanoma was excised, CyA had to be continued because of the severity of the patient's underlying disease and no recurrence of the malignancy was observed. Some points regarding the potential development of MM in association with CyA therapy are worth mentioning. First, other forms of immunosuppression, namely radiation therapy and antithymocyte globulin, have been reported to increase the risk for the development of MM up to 10·7-fold.[6] Second, MM are certainly not only more frequent but apparently also more aggressive in patients treated with conventional immunosuppressive agents than in patients treated with CyA.[4,8,9]

The authors seem to have misinterpreted the data published by Penn,[9] who showed that lymphomas were the most frequent cancers developing in patients treated with CyA (58 lymphomas, 142 tumours in total) while in patients treated with 'conventional immunosuppression' 319 lymphomas out of 2598 tumours developed. It is evident that no incidence figures can be derived from these data. Therefore, indirect approaches have been made to calculate the risk and/or incidence for patients receiving CyA or other immunosuppressive agents. It appears that the overall risk for the development of malignancies is not higher in CyA than in 'conventionally' treated patients.[10]

In conclusion, we cannot exclude that CyA may have contributed to the development of MM in the patients described by Mérot *et al.*,[1] but we hope that our comments will help the reader to see these two cases in the appropriate perspective.

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References

1 Mérot Y, Miescher PA, Balsiger F *et al*. Cutaneous malignant melanomas occurring under cyclosporin A therapy: a report of two cases. *Br J Dermatol* 1990; 123: 237–9.
2 Silverberg E, Ljbera J. Cancer Statistics 1986 (Holleb AJ, ed). CA: *A Cancer J Clinicians* 1986; 36: 5–37.
3 Kinlen LJ, Sheil AGR, Peto J, Doll R. Collaborative United Kingdom–Australian study of cancer in patients treated with immunosuppressive drugs. *Br Med J* 1979; 2:1461–6.
4 Sheil AGR, Flavel S, Disney APS *et al*. Cancer incidence in renal transplant patients treated with azathioprine or cyclosporin. *Transplant Proc* 1987; 19: 2214–16.
5 McLelland J, Rees, A, Williams G, Chu T. The incidence of immunosuppression-related skin disease in long-term transplant patients. *Transplantation* 1988; 46: 871–4.
6 Witherspoon RP, Fisher LD, Schock G *et al*. Secondary cancer after bone marrow transplantation of leukemia or aplastic anemia. *N Engl J Med* 1980; 321: 784–9.
7 Penn I. Cancer after cyclosporin therapy. *Transplant Proc* 1988; 20: (Suppl. 1): 276–9.
8 Penn I. Cancers following cyclosporin therapy. *Transplant Proc* 1987; 19: 2211–13.
9 Penn I. Cancer following cyclosporin therapy. *Transplantation* 1987; 43: 32–5.
10 Cockburn ITR, Krupp P. The risk of neoplasm in patients with cyclosporin A. *J Autoimmun* 1989; 2: 723–31.

ADONIS 000709639100150C

An unusual presentation of pseudoxanthoma elasticum with multiple comedones on the neck

Sɪʀ, We report a case of multiple comedones appearing on the neck of a patient with pseudoxanthoma elasticum (PXE).

A 32-year-old Japanese woman presented with cutaneous lesions that had been present for 28 years. She also had angina from the age of 15. There were many small yellowish papules arranged in a reticulate pattern on both sides of the neck, the axillae, cubital fossae and in both groins. These plaques had a 'cobblestone' appearance. There were many comedones present in the posterior cervical regions (Fig. 1) and most were open and randomly distributed. Angioid streaks were seen on ophthalmological examination. There was no family history of similar skin disease.

A biopsy from the left cervical region showed swollen and irregularly clumped elastic fibres in the upper and mid-dermis and von Kossa's stain showed the presence of calcium deposits along these fibres. An open comedo that contained many fragments of hair in its cavity was also seen (Fig. 2).

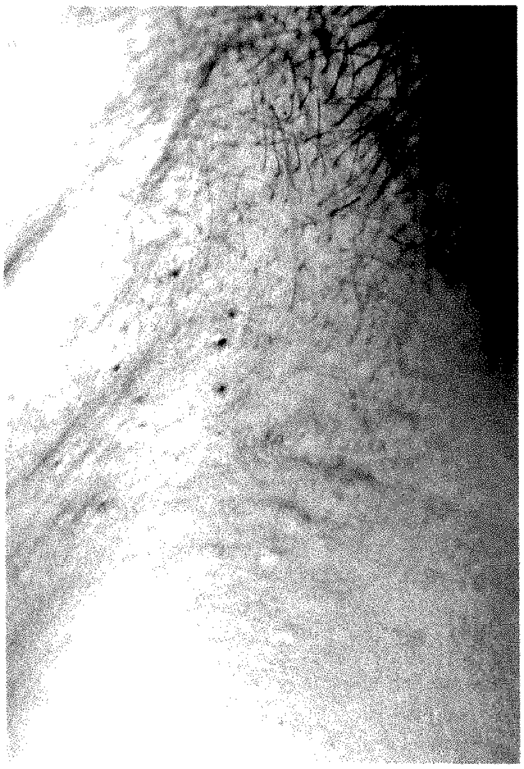

Figure 1. Comedones on the neck of a patient with pseudoxanthoma elasticum.

Laboratory investigations were all normal, but cardiac catheterization showed obstruction of the left anterior descending artery with marked stenosis of the left circumflex artery and a diffuse stenosis of the right coronary artery.

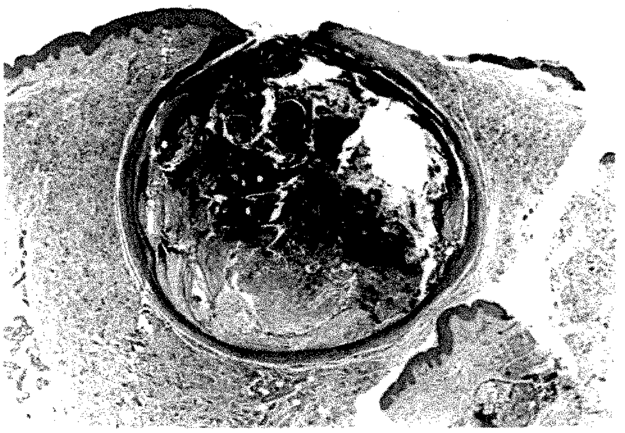

Figure 2. Skin biopsy from the neck showing fragmented elastic fibres and an open comedo with many hair fragments in the cavity (haematoxylin and eosin, × 100).

The multiple comedones seen in our patient are an unusual feature in PXE with only a few cases having been previously reported.[1-3] We consider that the degeneration of elastic fibres in the upper dermis is a factor in the development of these comedones, as in solar elastosis.

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References

1 Hartman A, Hartman-Visser SR. Pseudoxanthoma elasticum with extensive comedo formation. *Dermatologica* 1977; **154:** 318–19.

2 Dupre A, Bonafe JL, Christol B *et al.* Lesions acneiformes induites par le pseudoxanthome elastique. *Ann Dermatol Venereol* 1979; **106:** 481–7.

3 Heid E, Eberst E, Lazrak B, Basset A. Pseudoxanthome elastique et lesions acneiformes. *Ann Dermatol Venereol;* 1980; **107:** 569–71.

Aᴅᴏɴɪs 000709639100151P

The treatment of erythema multiforme major with systemic corticosteroids

Sɪʀ, The role of systemic corticosteroids in the treatment of erythema multiform major (Stevens–Johnson syndrome: EMm) remains controversial,[1,2] although the standard British textbook of dermatology recommends their use.[3] In a standard North American dermatology text the authors state 'the usefulness of systemic corticosteroids in erythema multiforme is not generally accepted' but then go on to suggest that 'it is . . . in our experience useful, to employ a short, tapering course of systemic steroids'.[4]

Clinical trials reported so far have either been a collection of case histories where systemic steroids have been used or not, or retrospective comparisons of cases treated with systemic steroids and those treated without. There is no published prospective controlled study comparing systemic steroids to placebo in the treatment of EMm. Ruiz-Malonado treated 39 patients with severe drug-associated EMm without using systemic steroids at all with excellent results.[5] Rasmussen,[1] in a retrospective study, compared 17 patients in whom systemic steroids were used with 15 patients in whom systemic steriods were avoided, and found the steroid-treated group suffered from more complications and spent longer in hospital. In another retrospective study with 51 paediatric patients, treatment with systemic steroids worsened the prognosis of EMm.[6] The drawbacks of these studies have been discussed at length by Renfro *et al*, who presented three case reports and concluded that high-dose systemic steroids should be given early in severe disease.[2] All authors stress the need for prospective studies to help resolve this therapeutic controversy.

We report the first controlled study of the use of systemic steroids in the treatment of EMm. Adult patients admitted to either Harare Central Hospital or Parirenyatwa Hospital in Harare, Zimbabwe, with a clinical diagnosis of erythema multiforme major were randomly assigned either prednisolone 30 mg daily, reduced by 5 mg each day, or matching placebo tablets. All patients were black Zimbabweans. In all cases their histology was consistent with the diagnosis of erythema multiforme, showing partial epidermal necrosis with some dermo-epidermal separation and a predominantly lymphocytic infiltrate in the superficial dermis. The mean age of the patients was 27·9 years (range 16–38 years). Four patients received prednisolone and five received placebo. All patients received the same regimen of oral hygiene and cutaneous lesions were treated with 1% silver sulphadiazine cream. In five cases there was a definite history of drug intake before the onset of the rash, two patients having ingested sulphonamides, two having taken caffeine tables and one having taken multiple analgesics. No patients in either group developed complications (fever, cutaneous infection, signs of chest infection, arthropathy, etc.). The prednisolone-treated group were discharged from hospital after an average 9·5 days, while the placebo-treated group were discharged after 8 days. Three out of four of the prednisolone-treated group, and one out of five of the placebo group were considered still to have active disease at 7 days.

The patients in this study all had relatively mild, uncomplicated EMm, and thus may not be representative of the complete spectrum of the disease. One of the reasons for this, and for our small numbers, was that the referring hospital physicians were unhappy that their patients may be randomized to the placebo group and would not permit their patients to participate in the study. This reflects the strong 'traditional' belief in the value of systemic steroids in the condition. There is no information on whether different racial groups suffering from EMm may have different outcomes. The conclusion from this small prospective controlled study of the use of systemic steroids in the treatment of erythema multiforme major confirms the other existing trial data that systemic steroids are of no value in its treatment and reaffirms the need for a definitive prospective study.

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References

1 Rasmussen JE. Erythema multiforme in children. Response to treatment with systemic corticosteroids. *Br J Dermatol* 1976; 95: 181–6.

2 Renfro L, Grant-Kels JM, Feder HM, Daman LA. Controversy: are systemic steroids indicated in the treatment of erythema multiforme? *Pediatr Dermatol* 1989; 6: 43–50.

3 Champion RH. Disorders affecting small blood vessels: erythema and telangiectasia. In: *A Textbook of Dermatology* (A Rook, DS Wilkinson, FJG Ebling, RH Champion, JL Burton, eds), 4th edn., Vol. 2. Oxford: Blackwell Scientific Publications, 1988; 1088.

4 Elias PM, Frotsch PO. Erythema multiforme. In: *Dermatology in General Medicine* (TB Fitzpatrick, AZ Eisen, K Wolff, IM Freedberg, KF Austen, eds), 3rd edn., Vol. 2. New York: McGraw Hill, 1987; 562.

5 Ruiz-Maldonado R. Acute disseminated epidermal necrosis types 1,2, and 3: Study of sixty cases. *J Am Acad Dermatol* 1985; 13: 623–35.

6 Ginsburg CM. Stevens–Johnson syndrome in children. *Pediatr Infect Dis* 1982; 1: 155–8.

ADONIS 0007096391001524

British Journal of Dermatology (1991) 124, 614.

Book Reviews

Nail Disorders. R.BARAN, J.BARTH, AND R.DAWBER (1991). London: Martin Dunitz. Pp. 188. Illustrations 193. ISBN 1-853-17004-6. Price £29.95.

This is a beautifully illustrated 'problem orientated' guide to aid the diagnosis of abnormal nails. As the book is structured around signs, not diseases, it is of much more value to the clinician faced with a patient with a nail problem that most previous 'nail' atlases. Chapter topics include, for example, abnormalities of nail configuration, modifications of nail surface, and nail colour changes.

Both the text and the photographs make for fascinating browsing—parrot beak nails, hook shaped deformities, 'worn down' and 'shiny' nails—every conceivable nail (even absent nails) are illustrated. The quality of reproduction is so good that 'it makes your nails hurt just to look at them', according to an uninvited reviewer looking over my shoulder. New or forgotten entities abound—I shall look out for BDD or blistering distal dactylitis—a variant of a streptococcal skin infection over the anterior foot pad of the distal phalanx of the finger and parakeratosis pustulos (p. 133) was also new to me. Sex even creeps into this nail book—apparently primary extragenital cutaneous gonorrhoea may present with a fingertip abscess.

Does 'dolichonychia' (long nails) really exist as an entity or are our eminent triple authors having us on? In this condition the 'length of the nail is much greater than the width'; I wonder what ratio is 'abnormal', as Figure 1.21 looks remarkably normal. In the following chapter Figures 2.1 and 2.3 are said to show 'age-related' longitudinal lines, but is there really any evidence that these changes are related to any ageing process?

There is a useful chapter giving some helpful hints concerning treatment of common nail conditions. However, the disability suffered by someone with lichen planus of the nails would surely have to be immense to justify treatment with systemic steroids for up to one year (p178). It is amazing to see evening primrose oil suggested for yet another condition—brittle nails—though the authors do say that there is no objective proof of its efficacy. Does methotrexate really slow nail growth rates 'by up to 100% less than normal rates' (p. 174); surely methotrexate does not stop nail growth completely.

This is an excellent book which can be recommended to all involved in caring for diseases of the hands and feet: its practical approach will for example make it very useful to chiropodists. Although the authors imply in the foreword that the book is not specifically aimed at dermatologists, I would recommend it for dermatology departments as a useful parallel book to the 1984 Baran and Dawber publication. When faced with an odd nail in the clinic, you will probably reach for this book first.

A final mystery remains. Is the traumatic subungual haematoma of the great toes of an anonymous marathon runner (Fig 2.23) a self-portrait of the toes of one of the authors? I think we should be told.

A.Y.FINLAY

Skin Immune System (SIS). Edited by J.D.Bos (1990). Boca Raton, Florida: CRC Press, Inc. Pp 501. ISBN 0-8493-4945-1. Price £120.

The editor's stated aim is that this book should represent a distillation of the latest information on the skin immune system, which would otherwise only be obtainable by consulting original articles. This he has achieved this by commissioning chapters from a panel of distinguished contributors. Study of the skin immune system in this detail is not of universal interest, and the book must be aimed primarily at investigators in the field.

Subjects covered in the first three sections include the overall organization of skin-associated lymphoid tissues, the role of adhesion molecules in inflammatory cell recruitment into the skin, the individual cellular constituents of the skin immune system, humoral skin immunity, and epidermal cytokines. The fourth section relates to alterations found as a result of specific dermatological diseases, immunodeficiency states, HIV-1 infection, photoirradiation, and pharmacological manipulation. The authors in this last section have been given a more difficult task, because it is difficult to compress the great amount of available data into a brief chapter.

Although the book was published in 1990 the preface is dated February 1989, and, as is inevitable with books of this nature, the field is advancing so rapidly that some recent developments have not been included. The nature of the content and the price at £120 means that this is not a book which the average dermatologist will be investing in. The investigator with special interest in the immunology of the skin will need to follow the literature and may also think twice before ordering a personal copy. Nevertheless, it does represent a comprehensive summary of the 'state of the art', and as such certainly deserves a place is a reference text in the departmental library.

S.M.BREATHNACH

British Journal of Dermatology (1991) **124**, 615.

News and Notices

2nd Congress of the European Academy of Dermatology and Venereology (EADV), 10–13 October 1991, Athens

The 2nd Congress of the European Academy of Dermatology and Venereology (EADV) will be held 10–13 October in Athens. The Congress will be organized by the EADV in cooperation with the Department of Dermatology of the University of Athens. For further information contact: the Congress Organizers and Secretariat, 24 Harilaou Trikoupi Street, Athens 106 79, Greece. Telephone 3609511; Fax (01) 3607962.

Omega-3 fatty acids: metabolism and biological effects International symposium 15–19 September 1992, Oslo, Norway

An international scientific symposium will be held on Omega-3 fatty acids: their metabolism and biological effects at the Oslo Plaza Hotel, 15–19 September 1992. Plenary sessions with invited speakers and parallel sessions on specific topics, as well as poster sessions, will be held. The deadline for submission of abstracts is 31 March 1992. For further information, contact: Professor Chr A.Drevon, Institute for Nutrition Research, University of Oslo, P.O. Box 1117, Blindern, N-0317 Oslo 3, Norway.

University of Nijmegen

Dr P.D.Mier has been appointed as an extraordinary Professor in Biochemistry of the Skin with emphasis on psoriasis at the University of Nijmegen.

The Skin Disease Research Fund

Applications are invited for research grants from The Skin Disease Research Fund and the deadline for submission is 2 September 1991. Only the Fund's own forms will be accepted and these can be obtained from: the Secretary, The Skin Disease Research Fund, 3 St Andrew's Place, London NW1 4LB. Telephone 071-935-8576.

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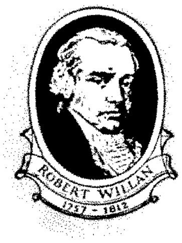

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gram(s)	g
kilogram(s)	kg
milligram(s) (10^{-3}g)	mg
microgram(s) (10^{-6}g)	μg
nanogram(s) (10^{-9}g)	ng
picogram(s) (10^{-12}g)	pg
hours(s)	h
minute(s)	min
centimetre(s)	cm
second(s)	s
cubic millimetre(s)	mm^3
millimetre(s)	mm
millicurie(s)	mCi
milliequivalent	mmol
molar	$mol\ l^{-1}$
millilitre(s)	ml
gravitational acceleration	*g*
micrometre(s)	μm
per cent	%
isotopic mass number placed as	^{131}I

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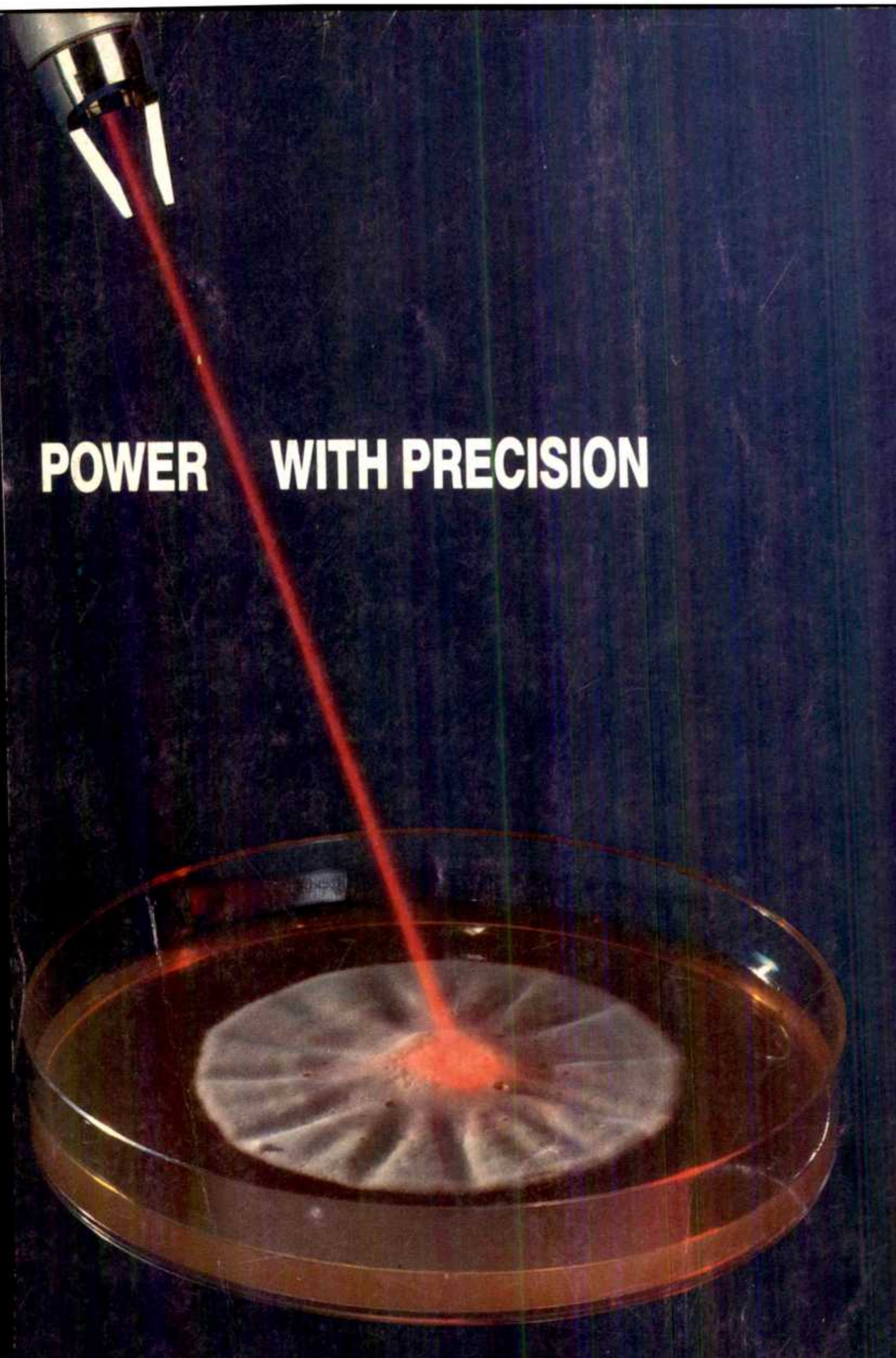

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References 1. Clayton YM. Clin Exp Dermatol, 1989, **14**, 2, 101-3. 2. Ryder NS. Antimicrob Agents Chemother, 1985; **27**, 252-6. 3. Schuster I. Xenobiotica, 1985, **15**, 529-46. 4. Schuster I, Ryder NS. J Dermatol Treat, 1990, **1**, (Suppl 2), 7-9. 5. Villars V, Jones TC. J Dermatol Treat, 1990, **1**, (Suppl 2), 33-8. 6. Savin R. Clin Exp Dermatol, 1989, **14**, 2, 116-19. 7. White JE, Perkins P, Ev[illegible] EGV. Br J Dermatol, 1990, **123**, (Suppl 37), 30.

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